Southern Forests: a Journal of Forest Science
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tsfs20

Allelopathic effects of invasive Eucalyptus camaldulensis on germination and early growth of four native species in the Western Cape, South Africa

Sheunesu Ruwanza[a], Mirijam Gaertner[a], Karen J Esler[b] & David M Richardson[a]

[a] Centre for Invasion Biology, Department of Botany and Zoology, Stellenbosch University, Stellenbosch, South Africa

[b] Centre for Invasion Biology, Department of Conservation Ecology and Entomology, Stellenbosch University, Stellenbosch, South Africa

Published online: 03 Oct 2014.

To cite this article: Sheunesu Ruwanza, Mirijam Gaertner, Karen J Esler & David M Richardson (2015) Allelopathic effects of invasive Eucalyptus camaldulensis on germination and early growth of four native species in the Western Cape, South Africa, Southern Forests: a Journal of Forest Science, 77:2, 91-105, DOI: 10.2989/20702620.2014.965985

To link to this article: http://dx.doi.org/10.2989/20702620.2014.965985

Southern Forests 2015, 77(2): 91–105

SOUTHERN FORESTS
ISSN 2070-2620 EISSN 2070-2639
http://dx.doi.org/10.2989/20702620.2014.965985

Allelopathic effects of invasive *Eucalyptus camaldulensis* on germination and early growth of four native species in the Western Cape, South Africa

Sheunesu Ruwanza[1]*, Mirijam Gaertner[1], Karen J Esler[2] and David M Richardson[1]

[1] *Centre for Invasion Biology, Department of Botany and Zoology, Stellenbosch University, Stellenbosch, South Africa*
[2] *Centre for Invasion Biology, Department of Conservation Ecology and Entomology, Stellenbosch University, Stellenbosch, South Africa*
** Corresponding author, e-mail: ruwanza@yahoo.com*

***Eucalyptus camaldulensis* Dehnh. (red river gum; Myrtaceae) is an invasive tree in riparian habitats of the Western Cape, South Africa, where it replaces indigenous vegetation and affects ecosystem functioning. These invasions lead to changes in river geomorphology and reduction in stream flow. The mechanisms that drive these effects are poorly understood. The potential for allelopathic effects of aqueous extracts of *E. camaldulensis* tissues and of soil and litter collected beneath *E. camaldulensis* trees on the germination and seedling growth of four selected native plant species was investigated in a greenhouse experiment. Soils collected beneath *E. camaldulensis* trees were used in three treatments: untreated soils, sterilised soils and sterilised soils overlaid with a eucalypt litter layer. In addition, soils collected from underneath native species were used in two treatments: untreated soils and soils overlaid with a eucalypt litter layer. All soil treatments were watered with three *E. camaldulensis* leaf, bark and root aqueous treatments. Compounds present in the aqueous extracts and fresh samples were identified using gas chromatography. Soil and aqueous treatments showed varying effects on germination and seedling growth of the four native species. Germination and seedling growth of *Olea europaea* subsp. *africana* and *Dimorphotheca pluvialis* were significantly reduced by *E. camaldulensis* root and bark aqueous extracts as well as by the soils treatments. The addition of eucalypt litter to native and sterilised soils reduced shoot and root growth of all four native species. Compounds such as α-phellandrene, eucalyptol, *p*-menth-1-en-8-ol and α-pinene, which have the potential to inhibit germination and plant growth, were identified in *E. camaldulensis* aqueous extracts and fresh samples. Although the methods applied in this study had limitations (e.g. lack of control treatment to litter addition), the results provide an additional motivation to prioritise removal of invasive *E. camaldulensis* stands from riparian ecosystems. Restoration initiatives should target native species that are not negatively affected by allelopathy.**

Keywords: allelopathy, biological invasions, germination, invasive plant species, phenolic compounds, tree invasions

Introduction

Many riparian ecosystems in South Africa, particularly in the Western Cape, have been invaded by alien tree species (Richardson and van Wilgen 2004). Invasive alien trees outcompete indigenous vegetation and affect key ecosystem functioning (Ehrenfeld 2003) and services provided by riparian systems (Richardson et al. 2007; Holmes et al. 2008). Invasion by Australian eucalypts (mainly *Eucalyptus camaldulensis* Denh.) has transformed long stretches of the Western Cape's Berg River and the lower reaches of the Sonderend River (Forsyth et al. 2004; Ruwanza et al. 2013a, 2013b). To reduce the undesirable effects of alien tree invasions in these riparian systems, large-scale mechanical control has been conducted by the Working for Water programme – a national agency created in 1995 to coordinate invasive plant management. This programme strives to protect and maximise water resources and enhance ecosystem health, while creating jobs for marginalised people (van Wilgen et al. 1998). The programme has been successful in many situations (Turpie et al. 2008), but in some cases the clearing operations have resulted in secondary invasions of the same or other alien species (Galatowitsch and Richardson 2005) and have exacerbated rather than alleviated ecosystem degradation (Richardson and Gaertner 2013). Fundamental reasons for the failure of native species to recover, proliferate and dominate communities after alien clearing are poorly understood. Several factors, including the allelopathic legacy effect in soils (Grman and Suding 2010) and its related interactions with recruiting native species, have been associated with the failure of native species to recover following the clearing of alien plants. Allelopathy has long been known to influence plant–plant interactions in many communities (Milchunas et al. 2011). An improved understanding of allelopathy is needed to guide alien plant management programmes and to improve the efficiency of interventions that aim to reintroduce native species as part of restoration.

Allelopathy has been suggested as a mechanism whereby certain alien species, e.g. *Eucalyptus urophylla* S.T.Blake (Zhang and Fu 2009) and *Acacia mearnsii* De Wild (Fatunbi et al. 2009), gain dominance in invaded ecosystems (Hierro and Callaway 2003). This phenomenon involves chemically mediated interference between plants, whereby secondary

compounds produced by one species directly or indirectly (e.g. by affecting soil biota) suppress the growth and fitness of other species (Inderjit and del Moral 1997; Hierro and Callaway 2003; Inderjit et al. 2011). Allelopathic effects have been reported to contribute to the success of several plant invaders, including *Eucalyptus* species (Khan et al. 2008; Zhang et al. 2010). In some forest plantations large areas of the soil surface beneath *Eucalyptus* canopies remain completely bare or have only sparse vegetation (El-Darier 2002; Fikreyesus et al. 2011). This phenomenon has been linked to allelopathy and the capacity of eucalypts to render soils unfavourable for many other plant species (El-Darier 2002). It is reported that *Eucalyptus* tissues, litter leachates and affected soils contain phenolic compounds that are detrimental to the germination and growth of other plant species (Sasikumar et al. 2001; Zhang et al. 2010). In a laboratory experiment, Khan et al. (2008) reported that aqueous extracts of *Eucalyptus camaldulensis* leaves were detrimental to the germination and seedling growth of wheat, barley and maize. However, very few studies have investigated the effects on germination and seedling growth of non-crop plants. Although allelopathy has been reported to inhibit the growth of seedlings of many species, few studies have shown enhanced plant growth, which is triggered by low-dose allelochemicals, a process called hormesis (Duke et al. 2006). Hormesis was first described by Southam and Erlich (1943) to explain the effect of an oak bark compound that promoted fungal growth at low doses, but strongly inhibited it at higher doses.

Recent studies have shown that some invasive plants have the potential to leave an allelopathic 'legacy effect' in the soils even after they have been removed (Siemens and Blossey 2007). Few studies have examined the length of time that *Eucalyptus* allelochemicals remain in the soil. May and Ash (1990) suggested that allelochemicals in soil and litter are easily washed out by water once the invader has been removed, resulting in a minimal legacy effect. In contrast, Gabriel (1975) reported that birch seedling survival and growth was adversely affected for five years in nursery soil formerly occupied by black walnut. The latter study, although not dealing with *Eucalyptus*, indicates that soils may retain allelopathic compounds or pathogens long after the invader has been removed. Should allelopathy persist in *Eucalyptus*-invaded sites, germination and survival of native seeds and seedlings may be inhibited on cleared areas (assuming absence of other recruitment limitations), and this may hamper efforts to restore the site by reintroducing native plant species.

Most studies on the allelopathic effects of *Eucalyptus* species have focused on the effect of leaf and litter extracts of the plant, and little attention has been given to other potential allelochemical sources of *Eucalyptus* (Singh et al. 2005; Bagavathy and Xavier 2007). Additional possible allelopathic sources of *Eucalyptus* were investigated, including root and litter leachates and affected soils, so as to detect the origin of any allelopathic effect and to analyse the effects of these sources on native species.

Prior research on allelopathy has been conducted under laboratory settings (Willis 1985; Sasikumar et al. 2001), which limits the ability to infer the ecological relevance of the results for native plant and soil communities (Inderjit 2001; Zhang and Fu 2009). Field and greenhouse studies of allelopathic effects under natural or semi-natural conditions are necessary for investigating the holistic allelopathic potential of plants (Jose et al. 2006; Zhang and Fu 2009). For this reason, a greenhouse study under semi-natural (ambient) conditions was conducted to investigate the allelopathic potential of eucalypts on native plants.

The allelopathic effects of *E. camaldulensis* aqueous extracts (leaf, bark and root tissues) were investigated, and soil and litter collected underneath the tree canopy, on the germination and growth of four native species. The hypothesis that was tested was that *E. camaldulensis* leaf, bark and root tissues as well as soil and litter collected underneath the tree canopy would reduce the germination and seedling survival of native species in invaded riparian communities. Allelopathic compounds released by *E. camaldulensis* aqueous extracts and fresh samples that had the potential to affect growth of native species were also investigated. The results are used to formulate strategies to optimise the recovery of native plant species following the removal of invasive *Eucalyptus* stands.

Methods and materials

Sampling sites

Project sites were on the banks of the Berg River in the Western Cape, South Africa, which is c. 294 km long with a catchment area of c. 7 715 km^2 (mostly used for agriculture) and flows into the Atlantic Ocean at Velddrif (de Villiers 2007). Sections of the Berg River are heavily invaded by alien trees, mainly *E. camaldulensis*, with less abundant stands of other species, notably *Acacia longifolia* (Andrews) Willd., *A. mearnsii* and *Populus* species (Geldenhuys 2008). Invasion of the Berg River by *E. camaldulensis* started about 50 years ago (Geldenhuys 2008). The study focused on a heavily invaded section between the source in Franschhoek (33°54′16.55″ S, 19°03′17.50″ E) and Hermon (33°26′06.64″ S, 18°57′22.69″ E) (for details, see Tererai et al. 2013). This specific section was selected as this allelopathy experiment formed part of a bigger restoration project that was conducted along the Berg River to control alien species and to reintroduce native species (Ruwanza et al. 2013a, 2013b); these results can therefore be applied to future restoration experiments.

The geology of the upper Berg River catchment is dominated by sandstone and quartzites of the Cape supergroup, whereas the rest of the catchment is underlain by Cape granites and Malmesbury Shales (de Villiers 2007). The catchment is characterised by nutrient-poor lithologies, but some areas consist of deep alluvial 'flood plains' with fertile sediments (de Villiers 2007). River flow peaks during the winter rainy season, from June to August, with rainfall averaging between 300 and 600 mm per annum (Mucina and Rutherford 2006).

Soil collection

Soil samples (28 cm wide × 30 cm long × 10 cm deep) were collected along the river in autumn (March 2011) from one natural (N = 40) (i.e. not invaded by *E. camaldulensis*) (33°26'46.83″ S, 18°57'27.72″ E) and three *E. camaldulensis* invaded (N = 60) (33°26'58.56″

S, 18°57'11.47″ E) sites and placed into plastic trays of similar above-mentioned dimensions. Only one natural site could be sampled as the river was heavily invaded by eucalypts with only a few remaining natural patches. This was also the only available natural site in close proximity to the invaded sites. All sites were approximately 20 m long × 15 m wide and were less than 100 m apart, with similar soil type and soil chemical properties (Tererai 2012). Soil samples were excavated near *E. camaldulensis* trunks where the highest concentrations of allelochemicals are likely to occur. Of the 40 samples from natural sites, 20 were allocated as control soils (referred to as native soils) and the other 20 were overlaid with an *E. camaldulensis* litter layer (referred to as native+litter soils). Of the 60 soil samples taken from *E. camaldulensis* invaded sites, 20 were retained as allelopathy-contaminated soils (referred to as stand soils), 20 were sterilised (referred to as sterilised soils) for 72 h at 200 °C, and the remaining 20 soils were sterilised and overlaid with a litter layer (referred to as sterilised+litter soils). The purpose of soil sterilisation was to eliminate soil biota (i.e. microbial communities) that could have been stimulated by accumulating *Eucalyptus* compounds in the soil (Jairus et al. 2011).

The collected soils were sieved through a 2 mm mesh before placing into replicated plastic trays (28 cm wide × 30 cm long × 10 cm deep) for the various treatments. Litter was collected from underneath the same *E. camaldulensis* stands (predominantly *E. camaldulensis* leaves, and twigs). It was first air dried, then chopped into smaller pieces before being overlaid 20 mm thick on top of the relevant treatment soils (Behera and Sahani 2003).

Eucalyptus camaldulensis *aqueous extracts*

Fresh *E. camaldulensis* leaf, bark and root material was collected in invaded stands at the same sites where soils were collected. Roots were collected by digging up living *E. camaldulensis* plants to a depth not exceeding 1 m. Root length ranged from 10 cm to 1 m, whereas root diameter averaged 5 cm. Samples were collected every two weeks over the experimental period and were chopped into smaller pieces, soaked in tap water for 48 h (ratio 10 g herbage to 100 ml water) and stirred regularly (Mohamadi and Rajaie 2009; Haddadchi and Massoodi Khorasani 2006). The suspension was filtered to remove the herbage and the resulting solution and tap water (control) was used to water soils in the relevant treatments. All samples were used whilst fresh and the above-mentioned collection intervals were based on demand for irrigation water in the greenhouse.

Greenhouse layout

Soils were transported to a passively ventilated greenhouse at Stellenbosch University where air temperatures closely approximated those outdoors. The experimental design consisted of the collected soils (referred to as soil treatments) being watered with the above-mentioned aqueous extracts (referred to as aqueous treatments). Soils were arranged on four tables located at different positions, with each table containing 25 trays (each table with at least one soil treatment, i.e. native soils, native+litter soils, stand soils, sterilised soils and sterilised+litter soils; $n = 5$ for all treatments). The total numbers of trays were 100 (each soil treatment replicated five times). Tables and trays were rotated monthly to account for minor variations in air temperature and light intensity within the greenhouse. The four watering treatments of leaf, bark, root and tap water were then administered per table (Figure 1).

Plant species

Germination and seedling growth was tested for three native riparian tree species (*Vachellia karroo* (Hayne) Banfi & Galasso [*Acacia karroo* Hayne], *Olea europaea* subsp. *africana* (Mill.) P.S.Green, *Diospyros glabra* (L.) De Winter) and an annual, *Dimorphotheca pluvialis* (L.) Moench. The first three species are found along the Berg River and were selected as potential target species for active restoration; the annual species *D. pluvialis* was used as an indicator of how native annuals might respond to *E. camaldulensis* allelopathy. Seeds for these species were obtained from the Kirstenbosch Botanical Gardens, Cape Town, South Africa. Seeds from Kirstenbosch are usually collected from the few individual populations found in the garden. Eight seeds of each of the four native species were sown at depths of 5–10 mm during autumn (April 2011) into each of the 100 trays. The four species were interspaced at 7 cm and individual seed at 3.5 cm (Figure 1) to avoid interspecific below-ground interactions. All trays were watered twice a day (approximately 5 mm d^{-1}), monitored and weeded weekly to remove non-target species. The amount of water supplied to the soils was calculated to deliver the average daily amounts of rainfall (c. 5 mm d^{-1}) recorded by Ruwanza et al. (2012) during the rainy winter season at a nearby site. Light penetration in the greenhouse was not measured; however, the passively ventilated greenhouse allowed light penetration throughout the day.

Germination and seedling growth measurements

Seeds that germinated from the different water and soil treatments were counted on a monthly basis and expressed as percentage of the total seeds sown. Seedling height was measured monthly. After seven months, at the end of the experiment (late October 2011) all seedlings were excavated with their roots intact and root length and total dry biomass was measured.

Identification of compounds in* Eucalyptus camaldulensis *samples

Samples of *E. camaldulensis* leaf, bark and root aqueous extracts and of fresh leaves, bark and root samples were collected at the onset of the experiment and analysed for presence of organic compounds using gas chromatography at Stellenbosch University's Central Analytical Facility. The gas chromatography was performed with a Waters GCT Premier AS 2000 instrument coupled to a mass spectrometer, equipped with a HP5 column (25 m, 0.25 mm ID, 0.25 μm film thickness). Temperatures were set at 260 °C for both the injection (split injection ratio of 1:5) and the ion source temperature. Helium was used as the carrier gas (1 ml min^{-1}). The temperature ramp regime was initiated by heating at 40 °C for 5 min, followed by an oven ramp to 150 °C at 5 °C min^{-1}, and a second ramp of 10 °C min^{-1} to 280 °C. A mass scanning range of 35–650 m/z (perfluorotri-*N*-butylamine as

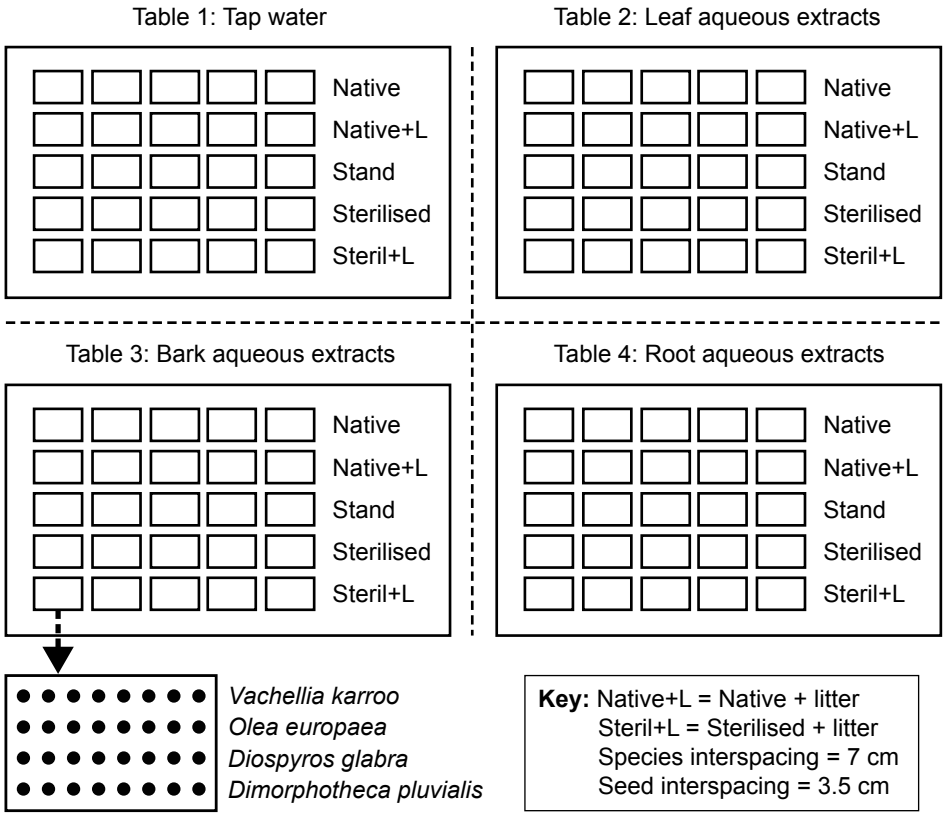

Figure 1: Greenhouse experimental design with four tables, each having different soil treatments and aqueous treatments. Sown species are also listed

mass reference) was employed and mass spectra were recorded at 2 scans s^{-1}. The Xcalibur™ software bundle version 1.2 (Finnigan Corporation, San Jose, CA, USA, 1998) was used for tentative compound identification and, where possible, authentic standards [camphene, (1R)-(+)-camphor, β-caryophyllene, (1R)-(+)α-pinene, (−)-α-bisabolol (Sigma–Aldrich, Steinheim Germany) and (+)-3-carene; R-(+)-limonene (Fluka, Sigma–Aldrich)] were used to confirm the identified compounds.

Statistical analysis

To determine germination and seedling growth responses to both aqueous extracts and soil and litter treatments, all measured variables (seed germination [%], shoot height [cm], root length [cm] and total dry biomass [g]) were subtracted for all of the four native species from their relative control treatments (the assumption being that they had no allelopathic effect) and expressed the resultant recalculated values as a percentage (Table 1). Shoot height was measured monthly between September and November 2011 to allow for calculations of temporal variation in seedling growth. The method we adopted for calculating plant growth variations relative to the control is based on the response coefficient estimator of plasticity as suggested by Valladares et al. (2006). This method involves comparing observed plasticity on seedling growth in response to allelopathy (i.e. active plasticity) to observed plasticity on seedling growth without allelopathy (i.e. passive plasticity).

Calculations were based on the equation:

$$PRC = (RTV - RCV/RCV) \times 100$$

where PRC = percentage rate of change, RTV = recorded treatment value, and RCV = recorded control value.

Performance index was calculated based on amending the method used by Azimi et al. (2013) to calculate vigor index. Calculations were based on the equation:

$$PI = MTG \times MTB$$

where PI = performance index, MTG = mean total germination per pot, and MTB = mean total biomass per pot.

Since 100% germination was not recorded, all values were subtracted from the recorded values of seedlings that germinated in the control treatments. Assumptions of normality were tested using the Shapiro–Wilk and Kolmogorov–Smirnov tests. Given that some of the variables (e.g. seed germination and total dry biomass) did not satisfy these assumptions, data were log transformed prior to analysis. A generalised linear model two-factor analysis of variance (ANOVA) and multiple analysis of variance (MANOVA) were used to test interactions between the different water and soil treatments on seed germination, shoot height, root length and total dry biomass for the four different species. Where results were significant, Tukey's HSD unequal *n* test was done to determine variance at

Table 1: Percentage changes relative to aqueous treatment control (tap water) and soil treatment control (native soils) of measured germination, shoot height, root length and total dry biomass in four native species. Data are calculated percentages based on the equation PRC = (RTV − RCV/RCV) × 100 (see text for details). See Figure 2 and Appendix 1 for statistical calculations. Native+L = Native + litter, Steril+L = Sterilised + litter

Parameter/species	Aqueous treatment			Soil treatment			
	Leaf	Bark	Root	Sterilised	Stand	Native+L	Sterile+L
Germination (%)							
Dimorphotheca pluvialis	−6.50	−27.27	−29.89	−75.58	10.45	−63.96	−69.77
Diospyros glabra	4.05	−4.05	−7.43	10.32	2.39	−37.30	−14.29
Olea europaea	−35.53	−57.76	−36.64	8.59	56.83	−78.21	−68.77
Vachellia karroo	−7.79	−4.55	−4.48	−35.61	−16.37	−53.87	−49.77
Shoot height (cm)							
Dimorphotheca pluvialis	−27.15	−22.38	−30.57	−59.22	−25.82	−65.17	−47.69
Diospryros glabra	−16.76	−18.38	−16.22	−21.23	−17.65	−23.79	−25.58
Olea europaea	−7.26	−9.68	9.68	−22.70	−4.29	−45.40	−53.37
Vachellia karroo	−12.59	−17.78	−6.67	−22.97	−24.03	−11.66	−9.54
Root length (cm)							
Dimorphotheca pluvialis	−34.14	−33.77	−41.37	−51.68	−20.37	−48.65	−49.49
Diospyros glabra	−5.56	−5.48	−5.31	−8.09	−7.00	−16.11	−19.07
Olea europaea	−36.07	−21.72	−28.07	−40.34	4.48	−70.17	−63.62
Vachellia karroo	−12.40	−7.50	−2.20	−5.57	−9.41	−15.85	−16.14
Total dry biomass (g)							
Dimorphotheca pluvialis	−42.34	−40.80	−32.09	−61.27	−51.94	−62.13	−73.41
Diospryros glabra	−50.39	−59.06	−53.54	−46.21	−47.73	−58.33	−62.12
Olea europaea	−41.18	−52.94	−47.06	−60.87	−26.09	−91.30	−78.26
Vachellia karroo	−47.46	−57.63	−52.54	−46.77	−61.29	−64.52	−40.32

$P < 0.05$. Interactions between treatments for all measured variables were analysed using both ANOVA and MANOVA in STATISTICA version 10 (StatSoft, Tulsa, OK, USA, 2010).

Results

Seed germination

Aqueous and soil treatments showed varying effects on germination of the four native species. Two-factor analysis showed that watering with aqueous extracts significantly reduced the germination of *D. pluvialis* ($F_{1:3} = 3.12$, $P < 0.05$) and *O. europaea* ($F_{1:3} = 15.63$, $P < 0.05$; Figure 2, Table 2). Germination reductions were most evident after watering *D. pluvialis* with root-derived water (30% reduction) and *O. europaea* with bark-derived water (58% reduction) relative to watering with tap water, which was the control (Table 1).

Soils collected beneath *E. camaldulensis* stands significantly ($F_{1:3} = 15.08$, $P < 0.001$) reduced germination of only one native species, namely *A. karroo* by 16%, but enhanced germination of the other three species (Table 1, Figure 2). The addition of *E. camaldulensis* litter to both native and sterilised soils reduced germination of all four native species ($P < 0.05$). The effects of litter layer addition were greatest for *O. europaea* (78% reduction) where litter was added to native soils (Native+L) and in *D. pluvialis* (70% reduction) where litter was added to sterilised soils (Steril+L; Table 1). Germination reductions following soil sterilisation alone occurred for *V. karroo* (36% reduction) and *D. pluvialis* (76% reduction; Table 1, Figure 2).

Only *O. europaea* germination was significantly ($F_{3:12} = 3.13$, $P < 0.05$) reduced by the interaction between aqueous and soil treatments (Table 2). Additional results on germination of the four species are shown in Appendix 1. Generally, the effects of both aqueous extracts and soil treatments were more pronounced on *O. europaea*, whilst *D. pluvialis* was most affected by allelopathy after soil sterilisation.

Seedling development

The effects of both aqueous and soil treatments on seedling development were different for all four species (Table 2). Aqueous treatments significantly reduced shoot-height growth of *D. glabra* ($F_{1:3} = 8.38$, $P < 0.05$) and *D. pluvialis* ($F_{1:3} = 3.82$, $P < 0.05$; Table 2). Shoot-height reductions were more evident in *D. glabra* (18% reduction) after watering with bark-derived water and *D. pluvialis* (31% reduction) after watering with root-derived water (Table 1). Similarly, aqueous treatments significantly reduced shoot-height growth of all plants for the recorded three months of September to November (Table 3). Reductions of root length caused by aqueous treatments were significant in *V. karroo* ($F_{1:3} = 4.93$, $P < 0.05$), *D. pluvialis* ($F_{1:3} = 10.19$, $P < 0.05$) and *O. europaea* ($F_{1:3} = 3.33$, $P < 0.05$; Table 2). Root-length reductions were more evident in *V. karroo* (12% reduction) and *O. europaea* (36% reduction) after watering with leaf-derived water and in *D. pluvialis* (41% reduction) after watering with root-derived water (Table 1).

There were significant ($P < 0.05$) differences in shoot height and root length of all four species among the different soil treatments (Table 2). Shoot-height and root-length reductions caused by soil collected underneath *E. camaldulensis* stands (26% and 20%, respectively) and soil sterilisation (59% and 52%, respectively) was more evident for *D. pluvialis* (Table 1). Monthly temporal growth variations were significant for all the plants

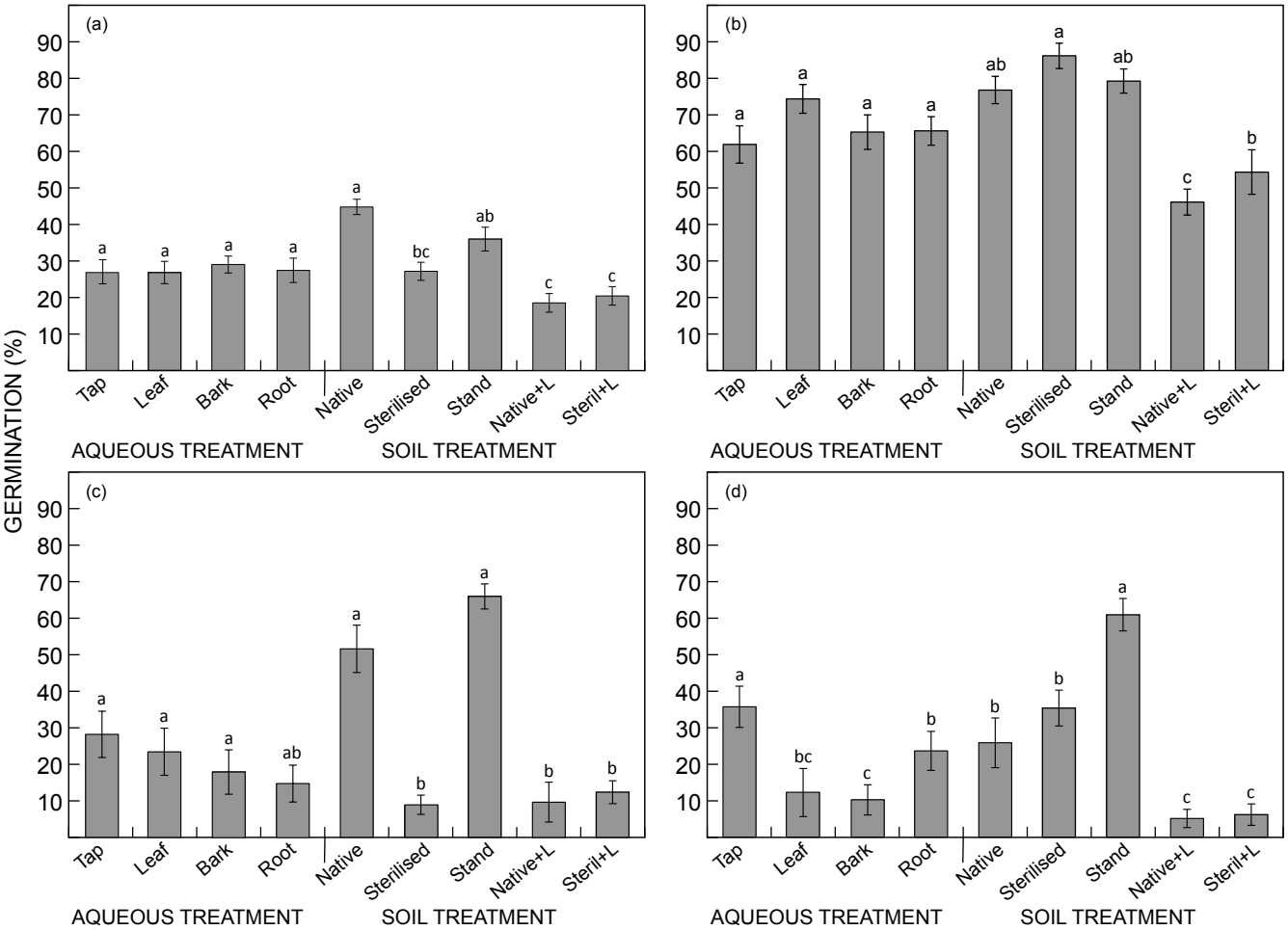

Figure 2: Effects of different *Eucalyptus camaldulensis* aqueous extracts and soil treatments collected from the Berg River in the Western Cape province, South Africa, on percentage germination rates (log_{10}) of (a) *Vachellia karroo*, (b) *Diospyros glabra*, (c) *Dimorphotheca pluvialis* and (d) *Olea europaea* in a greenhouse-based trial. Bars are means ± SD. Bars with different letter superscripts are significantly different at $P < 0.05$

Table 2: Generalised linear model ANOVA and MANOVA results for the effect of *Eucalyptus camaldulensis* aqueous extracts and soil treatments collected from the Berg River, Western Cape province, South Africa, on growth parameters (germination [log_{10}], shoot height, root length and total dry biomass [log_{10}]) of four native plant species in a greenhouse trial. Values in bold are significant at $P < 0.05$

Species/treatment		Germination (g)	Shoot height (cm)	Root length (cm)	Total dry biomass (g)	MANOVA		
	df	*F*-value	*F*-value	*F*-value	*F*-value	df	Wilks lambda	*F*-value
Dimorphotheca pluvialis								
Aqueous treatment	3	**3.12**	**3.82**	**10.19**	**6.03**	12	0.63	**3.15**
Soil treatment	4	**37.71**	**20.27**	**15.13**	**20.47**	16	0.16	**11.96**
Aqueous × soil treatments	12	1.75ns	0.91ns	1.29ns	0.64ns	48	0.53	1.10ns
Diospyros glabra								
Aqueous treatment	3	1.07ns	**8.38**	1.43ns	**28.74**	12	0.37	**7.70**
Soil treatment	4	**13.70**	**10.73**	**10.00**	**20.21**	16	0.22	**9.56**
Aqueous × soil treatments	12	1.17ns	0.65ns	0.88ns	0.85ns	48	0.55	1.05ns
Olea europaea								
Aqueous treatment	3	**15.63**	0.70ns	**3.33**	**5.66**	12	0.44	**6.08**
Soil treatment	4	**45.13**	**7.26**	**19.23**	**19.51**	16	0.15	**12.23**
Aqueous × soil treatments	12	**3.13**	1.29ns	**3.87**	1.19ns	48	0.28	**2.37**
Vachellia karroo								
Aqueous treatment	3	0.33ns	1.58ns	**4.93**	**11.23**	12	0.58	**3.73**
Soil treatment	4	**15.08**	**2.67**	**6.51**	**9.31**	16	0.31	**6.45**
Aqueous × soil treatments	12	0.63ns	1.20ns	**3.10**	**7.19**	48	0.30	**2.20**

ns = Non-significant

Table 3: Generalised linear model ANOVA results for the effect of *Eucalyptus camaldulensis* aqueous extracts collected from the Berg River in the Western Cape province, South Africa, on monthly shoot height of four riparian plant species (*Vachellia karroo*, *Diospyros glabra*, *Dimorphotheca pluvialis* and *Olea europaea*) in a greenhouse based trial. Columns with different letter superscripts are significantly different at $P < 0.05$

Species	September				October				November				ANOVA = *F*-values		
	Tap	Leaf	Bark	Root	Tap	Leaf	Bark	Root	Tap	Leaf	Bark	Root	Aqueous treatment	Months	Aqueous × months
Dimorphotheca pluvialis	14.31^{a}	11.13^{a}	12.23^{a}	10.23^{a}	19.79^{a}	15.61^{ab}	16.75^{ab}	14.33^{b}	22.21^{a}	16.18^{b}	17.24^{ab}	15.42^{b}	9.28***	49.83***	0.18ns
Diospyros glabra	1.80^{a}	1.22^{b}	1.17^{b}	1.24^{b}	2.78^{a}	2.15^{b}	2.07^{b}	2.13^{b}	3.70^{a}	3.08^{b}	3.02^{b}	3.10^{b}	61.38***	666.72***	0.27ns
Olea europaea	0.39^{b}	0.61^{a}	0.41^{b}	0.57^{ab}	0.85^{a}	1.03^{a}	0.81^{a}	1.01^{a}	1.24^{a}	1.15^{a}	1.12^{a}	1.36^{a}	7.52***	165.97***	0.22ns
Vachellia karroo	1.38^{a}	0.92^{b}	0.86^{b}	1.10^{ab}	2.13^{a}	1.57^{b}	1.45^{b}	1.76^{ab}	2.70^{a}	2.36^{b}	2.22^{b}	2.52^{ab}	18.50***	168.12***	0.11ns

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns = non-significant

Table 4: Generalised linear model ANOVA results for the effect of *Eucalyptus camaldulensis* soil treatments collected from the Berg River in the Western Cape province, South Africa, on monthly shoot height of four riparian plant species (*Vachellia karroo*, *Diospyros glabra*, *Dimorphotheca pluvialis* and *Olea europaea*) in a greenhouse based trial. Columns with different letter superscripts are significantly different at $P < 0.05$

Species	September					October					November					ANOVA *F*-values		
	Native	Sterilised	Stand	Native +L	Steril +L	Native	Sterilised	Stand	Native +L	Steril +L	Native	Sterilised	Stand	Native +L	Steril +L	Soil treatment	Months	Soil × months
Dimorphotheca pluvialis	19.24^{a}	4.62^{c}	11.43^{b}	5.32^{c}	8.52^{bc}	24.50^{a}	8.50^{c}	16.33^{b}	9.08^{c}	12.76b^{c}	29.30^{a}	11.99^{c}	21.81^{b}	10.24^{c}	15.38b^{c}	77.18***	46.08***	0.20ns
Diospyros glabra	1.89^{a}	1.22^{b}	1.32^{b}	1.09^{b}	1.17^{b}	2.90^{a}	2.12^{b}	2.25^{b}	1.96^{b}	2.07^{b}	3.91^{a}	3.08^{b}	3.22^{b}	2.98^{b}	2.91^{b}	58.40***	656.81***	0.22ns
Olea europaea	0.70^{a}	0.37^{b}	0.53^{ab}	0.43^{ab}	0.20^{b}	1.17^{a}	0.79^{bc}	1.02^{ab}	0.70^{bc}	0.53^{c}	1.63^{a}	1.26^{bc}	1.56^{ab}	0.89^{c}	0.76^{c}	30.72***	124.33***	1.24ns
Vachellia karroo	1.35^{a}	1.00^{ab}	0.85^{b}	0.94a^{b}	1.05^{ab}	2.01^{a}	1.58a^{b}	1.47^{b}	1.71^{ab}	1.82^{ab}	2.83^{a}	2.18^{b}	2.15^{b}	2.50^{ab}	2.56^{ab}	11.66***	157.85***	0.54ns

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns = non-significant

for the measured months of September to November (Table 4). The addition of litter to native and sterilised soils reduced shoot and root growth of all species, but these reductions were substantially higher in *O. europaea* and *D. pluvialis* (Table 1).

Only *V. karroo* and *O. europaea* root length was significantly ($F_{3:12}$ = 3.10, P < 0.05 and $F_{3:12}$ = 3.37, P < 0.05, respectively) reduced by the interactions between aqueous and soil treatments (Table 2). Additional results on shoot height and root length of the four species are shown in Appendix 1. Seedling growth effects of both aqueous and soil treatments were generally more pronounced on *D. pluvialis* and *O. europaea* than on the other two species.

Total dry biomass

Both aqueous and soil treatments significantly (P < 0.05) inhibited total dry biomass production of all four species (Table 2). Reductions in total dry biomass caused by aqueous treatments were observed in almost all species, with the highest total dry biomass reduction in *D. glabra* (59% reduction; $F_{1:3}$ = 28.74, P < 0.001) after watering with bark-derived water (Table 1). Similarly, reductions of total dry biomass caused by soil treatments were observed in all species (Table 1). Total dry biomass reductions caused by soil collected underneath *Eucalyptus* stands were highest for *V. karroo* (60% reduction; $F_{1:3}$ = 9.31, P < 0.001). Total dry biomass reductions caused by the addition of litter to native and sterilised soils were highest in *O. europaea* (91% and 78%, respectively; Table 1).

Only *V. karroo* total dry biomass was significantly ($F_{3:12}$ = 7.19, P < 0.05) affected by the interactions between aqueous and soil treatments (Table 2). Additional results on germination of the four species are shown in Appendix 1.

The effects of both aqueous and soil treatments were significant (P < 0.05) for germination/shoot height/root length/total dry biomass interactions for all the four species (Table 2). Two-way interaction between water × soil treatments for germination/shoot height/root length/total dry biomass was only significant in *V. karroo* (Wilks lambda = 0.30, $F_{12:48}$ = 2.20, P < 0.05) and *O. europaea* (Wilks lambda = 0.28, $F_{12:48}$ = 2.37, P < 0.05; Table 2). Effects of both aqueous and soil treatments on total dry biomass were evident on all species.

Performance index

Both aqueous and soil treatments had a significantly (P < 0.05) negative effect on growth performance of all the four species (Table 5). Overall plant performance for all four species was significantly (P < 0.05) high following watering the plants with tap water. Similarly, plants sown in soils collected underneath native species had higher growth performance than plants sown in any other soils. Two-way interaction between water × soil treatments for plant performance was only significant ($F_{(12:81)}$ = 6.83) for *V. karroo* (Table 5).

Compounds present in* Eucalyptus camaldulensis *samples

The dominant organic compounds were monoterpenoids, alkenes and phenolic compounds (Tables 6 and 7, Appendix 2). Most of the identified organic compounds were in leaf (28) aqueous extracts compared to bark (7) and root (5) aqueous extracts (Table 6). A single compound, 1-undecene, was identified only from root aqueous extracts and two compounds of thymoquinone and *p*-benzoquinone, 2,6-di-tert-butyl- were identified as distinct in root aqueous extracts. Four compounds, namely α-phellandrene, (+)-sabinene, eucalyptol and *p*-menth-1-en-4-ol, (R)-(−)-, were identified in all aqueous extracts (Table 6). Similarly, more compounds were identified in leaf (14) fresh samples than in bark (5) and root (4) fresh samples (Table 7). Two compounds, namely nonane and acetic acid, were present only in root fresh samples. Three compounds were found only in leaf fresh samples and two compounds in all leaf, root and bark samples (Table 7).

Discussion

Aqueous and soil treatments had variable effects on germination and seedling growth of the four study species. In most cases germination and seedling growth of the species were inhibited by aqueous and soil treatments. However, there were also instances where germination of plants was enhanced, for example for *D. pluvialis*, *O. europaea* and *D. glabra* in stand soils. Enhanced germination could be a result of hormesis (Duke et al.

Table 5: Generalised linear model ANOVA results for the effect of *Eucalyptus camaldulensis* aqueous extracts and soil treatments collected from the Berg River in the Western Cape province, South Africa, on the performance index of *Vachellia karroo*, *Diospyros glabra*, *Dimorphotheca pluvialis* and *Olea europaea* in a greenhouse-based trial. Columns with different letter superscripts and values in bold are significantly different at P < 0.05

Species	Aqueous treatment				Soil treatment					ANOVA $F_{(3;81)}$	ANOVA $F_{(4;81)}$	ANOVA $F_{(12;81)}$
	Tap	Leaf	Bark	Root	Native	Sterilised	Stand	Native+L	Steril+L	Aqueous treatment	Soil treatment	Aqueous × soil treatments
Dimorphotheca pluvialis	4.84^{a}	2.79^{b}	2.87^{b}	3.16^{b}	6.85^{a}	2.65^{b}	3.29^{b}	2.46^{b}	1.82^{b}	**6.07**	**20.62**	0.58ns
Diospyros glabra	0.16^{a}	0.08^{b}	0.06^{b}	0.07^{b}	0.16^{a}	0.09^{b}	0.09^{b}	0.07^{b}	0.06^{b}	**28.74**	**20.21**	0.84ns
Olea europaea	0.02^{a}	0.01^{b}	0.01^{b}	0.01^{b}	0.03^{a}	0.01^{b}	0.02^{b}	0.01^{b}	0.01^{b}	**6.36**	**19.46**	1.48ns
Vachellia karroo	0.07^{a}	0.04^{b}	0.03^{b}	0.04^{b}	0.08^{a}	0.04^{b}	0.03^{b}	0.03^{b}	0.04^{b}	**11.44**	**9.40**	**6.83**

ns = Non-significant

2006). This suggests that the effects of both treatments on germination are species specific, but more significant after germination, therefore affecting the seedling stage. Several other studies have shown that allelopathic effects of eucalypts are species specific (Sasikumar et al. 2001; Niakan and Saberi 2009), with effects being caused by specific mechanisms acting at the cellular or molecular level in the receiving plants (Niakan and Saberi 2009).

Effects of aqueous treatments on native species

The inhibitory effect of *E. camaldulensis* aqueous treatments on germination and seedling growth of some native species suggested that tissues of *E. camaldulensis* were allelopathic. Results for germination and growth of the four tested native species following aqueous treatments are consistent with other studies. Mohamadi and Rajaie (2009), Zhang and Fu (2009) and Zhang et al. (2010) showed decreased plant germination and growth after watering with *Eucalyptus* aqueous extracts. Although few studies have tested the allelopathic effects of *Eucalyptus* bark on native species, Schumann et al. (1995) showed that water extracts from both mulched leaves and branches of *E. grandis* suppressed seed germination and early seedling growth of three dicot and one monocot species.

Several studies have shown that leaf, bark and root tissues of certain *Eucalyptus* species produced phenolic acids and volatile oils that had deleterious effects on other plant species (Sasikumar et al. 2001). Germination of some species depended on α-amylase activity that regulates starch breakdown (Mohamadi and Rajaie 2009). Studies on *E. globulus* leaf leachates confirmed decreased α-amylase activity in seeds of finger millet (*Eleusine coracanta*), which resulted in inhibition of germination (Padhy et al. 2000). Research has also shown that, under allelopathic stress, the germination process is delayed or decreased (Gniazowska and Bogatek 2005) due to disruption of

Table 6: Major volatile organic components of *Eucalyptus camaldulensis* leaf, root and bark aqueous extracts used for watering native plants (identified by gas chromatography–mass spectrometry [GC-MS]). CF = Chemical formula, RT = experimental retention time (min) determined on the BP5 column, RI = experimental retention index, MW = molecular weight from GC–MS data

No.	Compound	CF	RT	RI	MW	Leaf	Root	Bark
1	α-Phellandrene	$C_{10}H_{16}$	9.33		136	*	*	*
2	(+)-4-Carene	$C_{10}H_{16}$	11.70		136	*	–	–
3	(+)-Sabinene	$C_{10}H_{16}$	12.12		136	*	*	*
4	Eucalyptol	$C_{10}H_{18}O$	12.20	1 059	154	*	*	*
5	1-Undecene	$C_{11}H_{22}$	14.32		154	–	*	–
6	3-Carene	$C_{10}H_{16}$	14.65	948	136	*	–	*
7	*p*-Menth-1-en-4-ol, (R)-(−)-	$C_{10}H_{18}O$	16.94	1 137	154	*	*	*
8	*p*-Menth-1-en-8-ol	$C_{10}H_{18}O$	17.44	1 143	154	*	–	–
9	Thymoquinone	$C_{10}H_{12}O_2$	19.11	1 340	164	–	–	*
10	3-Cyclohexen-1-one, 2-isopropyl-5-methyl-	$C_{10}H_{16}O$	19.23	1 130	152	*	–	–
11	Benzenemethanol, 4-(1-methylethyl)-	$C_{10}H_{14}O$	20.35	1 284	150	*	–	–
12	Aromadendrene, dehydro-	$C_{15}H_{22}$	24.36	1 396	202	*	–	–
13	Cycloisolongifolene, 8,9-dehydro-	$C_{15}H_{22}$	24.43	1 179	202	*	–	–
14	*cis*-(−)-2,4a,5,6,9a-Hexahydro-3,5,5,9-tetramethyl(1H) benzocycloheptene	$C_{15}H_{24}$	24.56	1 471	204	*	–	–
15	*p*-Benzoquinone, 2,6-di-tert-butyl-	$C_{14}H_{20}O_2$	24.75	1 633	220	–	–	*
16	Bicyclo[4.4.0]dec-5-ene, 1,5-dimethyl-3-hydroxy-8-(1-methylene-2-hydroxyethyl-1)-	$C_{15}H_{24}O_2$	25.28	1 933	236	*	–	–
17	(+)-Ledene	$C_{15}H_{24}$	25.44	1 419	204	*	–	–
18	Benzene, 1-methyl-4-(1,2,2 trimethylcyclopentyl)-, (R)-	$C_{15}H_{22}$	25.96	1 556	202	*	–	–
19	Neoisolongifolene, 8,9-dehydro-	$C_{15}H_{22}$	26.36	1 398	202	*	–	–
20	(−)-Spathulenol	$C_{15}H_{24}O$	27.41	1 536	220	*	–	–
21	7-Tetracyclo[6.2.1.0(3.8)0(3.9)]undecanol, 4,4,11,11-tetramethyl-	$C_{15}H_{24}O$	27.65	1 385	220	*	–	–
22	Varidiflorene	$C_{15}H_{24}$	27.76	1 419	204	*	–	–
23	χ-Himachalene	$C_{15}H_{24}$	27.97	1 499	204	*	–	–
24	Cycloisolongifolene, 8-hydroxy-, endo-	$C_{15}H_{24}O$	28.11	1 385	220	*	–	–
25	2-(4a,8-Dimethyl-1,2,3,4,4a,8a-hexahydro-2-naphthalenyl)-2-propanol #	$C_{15}H_{24}O$	28.64	1 580	220	*	–	–
26	α-Copaen-11-ol	$C_{15}H_{24}O$	28.85	1 377	220	*	–	–
27	τ-Cadinol	$C_{15}H_{26}O$	28.90	1 580	222	*	–	–
28	2(1H)Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-	$C_{15}H_{22}O$	30.18	1 673	218	*	–	–
29	Caryophyllene	$C_{15}H_{24}$	30.48	1 494	204	*	–	–
30	Spiro-1-(cyclohex-2-ene)-2′-(5′-oxabicyclo [2.1.0]pentane), 1′,4′,2,6,6-pentamethyl-	$C_{14}H_{22}O$	30.59	1 358	206	*	–	–
31	Tricyclo[5.1.0.0(2,4)]oct-5-ene-5-propanoic acid, 3,3,8,8-tetramethyl-	$C_{15}H_{22}O_2$	32.83	1 660	234	*	–	–

* Detected in *E. camaldulensis* leaf, root and bark solutions

Table 7: Major volatile organic components of *Eucalyptus camaldulensis* fresh leaf, root and bark samples used to prepare aqueous extracts for watering native plants (identified by gas chromatography–mass spectrometry [GC-MS]). CF = Chemical formula, RT = experimental retention time (min) determined on the BP5 column, RI = experimental retention index, MW = molecular weight from GC–MS data

No.	Compound	CF	RT	RI	MW	Leaf	Root	Bark
1	Nonane	C_9H_{20}	7.46		128	−	*	−
2	1,4-*p*-Menthadiene	$C_{10}H_{16}$	8.40	998	136	*	−	−
3	3-Octen-5-yne, 2,7-dimethyl-, (E)-	$C_{10}H_{16}$	8.61	912	136	*	−	−
4	Bicyclo[3.1.0]hex-2-ene, 4-methylene-1-(1-methylethyl)-	$C_{10}H_{14}$	9.10	879	134	*	−	−
5	Oxime-, methoxy-phenyl-_	$C_8H_9NO_2$	9.33	1 301	151	−	−	*
6	α-Pinene	$C_{10}H_{16}$	10.13	943	136	*	−	−
7	3-Carene	$C_{10}H_{16}$	13.22	948	136	*	−	−
8	1,6-Octadien-3-ol, 3,7-dimethyl-, acetate	$C_{12}H_{20}O_2$	14.69	1 272	196	*	−	−
9	Benzenemethanol, 4-(1-methylethyl)-	$C_{10}H_{14}O$	20.37	1 284	150	*	−	−
10	2,4,4-Trimethyl-3-(3-methylbutyl)cyclohex-2-enone	$C_{14}H_{24}O$	21.58	1 520	208	−	−	*
11	(−)-α-Elemene	$C_{15}H_{24}$	22.89	1 398	204	*	−	−
12	(−)-α-Gurjunene	$C_{15}H_{24}$	23.30	1 419	204	*	*	*
13	1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene-	$C_{15}H_{24}$	24.00	1 386	204	*	*	*
14	Bicyclo[4.4.0]dec-5-ene, 1,5-dimethyl-3-hydroxy-8-(1-methylene-2-hydroxyethyl-1)-	$C_{15}H_{24}O_2$	25.28	1 933	236	*	−	−
15	1H-2-Benzopyran-1-one, 3,4-dihydro-8-hydroxy-3-methyl-	$C_{10}H_{10}O_3$	26.68	1 674	178	−	−	*
16	Aristolone	$C_{15}H_{22}O$	30.21	1 574	218	*	−	−
17	Longipinocarvone	$C_{15}H_{22}O$	30.52	1 569	218	*	−	−
18	Vellerdiol	$C_{15}H_{24}O_2$	30.6	1 926	236	*	−	−
19	Acetic acid, tricyclo[3.3.1.1(3,7)]decylidene-, ethyl ester	$C_{14}H_{20}O_2$	30.62	1 431	220	−	*	−

* Detected in *E. camaldulensis* leaf, root and bark fresh samples

normal cellular metabolism, which then affects seedling emergency (Mohamadi and Rajaie 2009).

The leachates of *E. camaldulensis* have been shown to cause significant shoot and root reduction in crop species such as *Phaseolus vulgaris* and *Sorghum bicolor* (Mohamadi and Rajaie 2009). Plants that are rich in monoterpene hydrocarbons of α-phellandrene, α-pinene and myrcene and oxygenated monoterpenes of cryptone and terpinen-4-ol have been shown to be allelopathic as they have been reported to inhibit germination and radical elongation of other species such as wheat (Zahed et al. 2010) and herbaceous species, e.g. *Sinapis arvensis*, *Trifolium campestre*, *Lolium rigidium* and *Phalaris canariensis* (Amri et al. 2012). De Moral and Muller (1970) also showed that terpenes such as 1,4-*p*-menthadiene inhibit plant growth. Although the method used to identify major compounds in this study was not ideal as it does not detect essential phenolic compounds that are present as glycosides, major compounds of α-phellandrene, eucalyptol and *p*-menth-1-en-8-ol in solutions and α-pinene, bicyclo[3.1.0]hex-2-ene, 4-methylene-1-(1-methylethyl)- and 1,4-*p*-menthadiene in leaf samples could be identified. Previous studies by Grbović et al. (2010) and Sahin Basak and Candan (2010) also identified these major compounds in *E. camaldulensis* leaf samples.

Acetic acid, which was identified in root samples, is known to inhibit plant growth by damaging chromosome structure (Sugiyama et al. 2004). Other studies have shown that the inhibition of shoot and root growth by *Eucalyptus* may be linked to the presence of higher concentrations of terpenes and phenols such as chlorogenic, *p*-coumaryl quinic, gentistic and gallic acid (del Moral et al. 1978). These phenolic compounds might interfere with the phosphorylation pathway of plants and inhibit the activation of Mg^{2+} and ATPase activity (Sasikumar et al. 2001). Such interference will lead to decreased synthesis of total carbohydrates and proteins, cell division, mineral uptake and biosynthetic processes (Sasikumar et al. 2001). The above views are confirmed by studies that have reported decreased chlorophyll content after watering other species (mainly crop species) with *Eucalyptus* leaf leachates (Singh and Ranjana 2003; Mohamadi and Rajaie 2009). The reduction in chlorophyll content might be due to degradation of chlorophyll pigments or reduction in their synthesis and the action of flavonoids, terpenoids and other phytochemicals present in leaf leachates (Tripathi et al. 1999).

Effects of soil treatments on native species

The effects of soil treatments on native species were highly variable, with both increases on germination of *D. pluvialis*, *O. europaea* and *D. glabra* and reductions in germination for species grown in soils collected from *Eucalyptus* stands compared to native control soils. However, shoot height, root length and total dry biomass for all species were inhibited by *E. camaldulensis* soil treatments, and the addition of *E. camaldulensis* litter resulted in further inhibition. Studies that have examined allelopathic effects of soils underneath *Eucalyptus* stands have shown that *Eucalyptus* soils have variable effects (inhibitory and slightly stimulatory) on plants, especially crop plants such as maize, beans, watermelon and squash (Espinosa-García et al. 2008). Espinosa-García et al. (2008) showed that *Eucalyptus grandis* × *E. urophylla* was most inhibitory in upper layers of soil (A_0 horizon).

Soil beneath *Eucalyptus* trees has been reported to contain compounds that negatively affect plant growth (Espinosa-García et al. 2008). May and Ash (1990) and Khan et al. (2008) showed that these compounds can alter microbial communities, especially phytotoxic microorganisms that have the potential to negatively affect plant growth and microbial balance in the rhizosphere

(Souto et al. 2001). A monoterpene of eucalyptol (also called cineol), α-phellandrene and sabinene were identified in root samples. These volatile monoterpenes, especially eucalyptol, have been reported to inhibit growth of plant root and shoots by causing cork-screw-shaped morphological distortions as well as stress to photosynthesis, which result in reduced root growth and germination (Romagni et al. 2000).

The recorded increased germination of *D. pluvialis*, *O. europaea* and *D. glabra* in stand soils could be linked to hormesis (Duke et al. 2006). Although no study could be found that has shown *E. camaldulensis* allelochemicals to stimulate germination and growth of other plants, it is surmised that the species that showed increased germination on stand soils have the potential to develop physiological defence mechanisms that allow them to escape chemical stress (Duke et al. 2006). Surprisingly, shoot height, root length and total biomass of these species in the same soils was reduced compared to growth in soils collected underneath native stands.

Soils were sterilised to eliminate the effects of soil biota (i.e. microbial communities) that could have been stimulated by accumulating *Eucalyptus* compounds. Higher seed germination and seedling growth was expected in sterilised soils where biotic effects linked to allelopathy had been eliminated. However, all four study species showed reduced shoot and root growth in sterilised soils. The alternative explanation is that soil sterilisation eliminated all soil biota including those that have a positive effect on native species germination and growth (Razavi darbar and Lakzian 2007). Heating the soils to such a high temperature could also have increased soil water repellency (Coelho et al. 2005). Burning induces soil water repellency (Coelho et al. 2005), which reduces seed germination and seedling survival since water infiltration is reduced.

A key challenge in allelopathy studies is to separate plant growth effects caused by allelochemicals and those caused by soil nutrients (Jensen et al. 2001). An examination of basic soil-nutrient levels in the different soil treatments can help to determine whether any plant-growth effects are related to allelopathy or soil nutrients. Soil nutrients were not analysed as another study conducted in parallel with this one along the Berg River (Tererai 2012) showed no significant differences in soil-nutrient levels between areas invaded by *E. camaldulensis* and matched sites without invasive eucalypts. Allelopathic effects are therefore very likely the cause of the reduced plant growth observed in this experiment.

Another variable that could possibly have influenced our results is the physical effect of litter on germination and growth by modifying the soil environment (e.g. by changing soil temperature and moisture). With hindsight, this potentially confounded effect could have been eliminated or reduced by using native mulch so that observed effects could be unequivocally attributed to chemical rather than physical effects of the litter.

Implications of Eucalyptus camaldulensis *allelopathy for restoration*

These results suggest that *E. camaldulensis* has important allelopathic effects on native species in riparian plant communities in the Western Cape, South Africa. This provides additional motivation for management interventions to remove this invasive species from these ecosystems. Allelopathic legacy effects might persist after *Eucalyptus* removal, which may necessitate additional interventions if the aim is to restore native plant communities.

These results also suggest that the four native species tested in this study cannot be used for restoration initiatives along the Berg River. It is suggested that native species that are already growing underneath *E. camaldulensis* should be used for restoration as they are able to persist under allelochemical conditions. For example, Holmes et al. (2008) suggested that areas with low invasion intensity might provide conditions for both natives and aliens to grow together. Since the sites had heavy invasion, none of the tested species occurred underneath eucalypts. However, future restoration studies along the Berg River should examine the allopathic response of understory natives in low invasion sites.

One option for neutralising the effects of allelochemicals to facilitate effective restoration is to transfer soil from uncolonised sites. Costs involved with this option are likely to make it unrealistic in most cases, but this approach may be justifiable in selected high-priority sites. An added advantage under this scenario would be that the full complement of species in the soil seed bank would be simultaneously introduced, which could fast-track revegetation. Further studies are needed to examine the extent of allelopathy neutralisation by soils transfer and its financial and other implications.

Although the methods applied in this study had several limitations, they allowed us to conclude that the invasive tree *E. camaldulensis* has the potential to release allelopathic chemicals that hinder the growth of some native species that are targeted for restoration. Further experiments to examine allelopathy effects that include effects of competition and soil-nutrient changes are needed. In addition, further research on potential allelopathy of alien species on native species in the Western Cape riparian systems should examine more native plant species so as to determine more comprehensively the overall potential impact and to identify species with good potential for use in restoration.

Acknowledgements — This work was funded by the DST-NRF Centre of Excellence for Invasion Biology (C·I·B) and the Working for Water (WfW) Programme through their collaborative research project on 'Research for Integrated Management of Invasive Alien Species'. SR also thanks the Oppenheimer Memorial Trust for additional funding (OMT Ref. 19351/01 and 19351/02). DMR acknowledges funding from the National Research Foundation (grant 85417). We thank the Central Analytical Facility at Stellenbosch University for running the gas chromatography analysis; four anonymous referees for valuable comments; the farmers in the upper Berg River catchment for permission to work on their land; Manfred Paulsen (WfW implementation manager) for liaising with WfW contractors; Suzaan Kritzinger-Klopper (Senior Technical Officer at the C·I·B) for technical support and assistance in liaising with landowners; and Farai Tererai for general assistance.

References

Amri I, Hamrouni L, Hanana M, Jamoussi B. 2012. Herbicidal potential of essential oils from three Mediterranean trees on

different weeds. *Current Bioactive Compounds* 8: 3–12.
Azimi R, Feizi H, Khajeh Hosseini M. 2013. Can bulk and nanosized titanium dioxide particles improve seed germination features of Wheatgrass (*Agropyron desertorum*). *Notulae Scientia Biologicae* 5: 325–331.
Bagavathy S, Xavier GSA. 2007. Effects of aqueous extract of *Eucalyptus globulus* on germination and seedling growth of sorghum. *Allelopathy Journal* 20: 395–401.
Behera N, Sahani U. 2003. Soil microbial biomass and activity in response to *Eucalyptus* plantation and natural regeneration on tropical soil. *Forest Ecology and Management* 174: 1–11.
Coelho COA, Laouina A, Regaya K, Ferreira AJD, Carvalho TMM, Chaker M, Naafa R, Naciri R, Boulet AK, Keizer JJ. 2005. The impact of soil water repellency on soil hydrological and erosional processes under *Eucalyptus* and evergreen *Quercus* forests in the Western Mediterranean. *Australian Journal of Soil Research* 43: 309–3198.
del Moral R, Muller CH. 1970. The allelopathic effects of *Eucalyptus camaldulensis*. *American Midland Naturalist* 83: 254–282.
del Moral R, Willis RJ, Ashton DH. 1978. Suppression of coastal health vegetation by *Eucalyptus baxteri*. *Australian Journal of Botany* 26: 203–219.
de Villiers S. 2007. The deteriorating nutrient status of the Berg River, South Africa. *Water SA* 33: 1–6.
Duke SO, Cedergreen N, Velini ED, Belz RG. 2006. Hormesis: is it an important factor in herbicide use and allelopathy. *Outlooks on Pest Management* 17: 29–33.
Ehrenfeld JG. 2003. Effects of exotic plant invasions on soil nutrient cycling processes. *Ecosystems* 6: 503–523.
El-Darier SM. 2002. Allelopathic effects of *Eucalyptus rostrata* on growth, nutrient uptake and metabolite accumulation of *Vicia faba* L. and *Zea mays* L. *Pakistan Journal of Biological Sciences* 5: 6–11.
Espinosa-García FJ, Martinez-Hernandez E, Quiroz-Flores A. 2008. Allelopathic potential of *Eucalyptus* spp. plantations on the germination and early growth of annual crops. *Allelopathy Journal* 21: 25–38.
Fatunbi AO, Dube S, Yakubu MT, Shabalala T. 2009. Allelopathic potential of *Acacia mearnsii* De Wild. *World Applied Sciences Journal* 7: 1488–1493.
Fikreyesus S, Kebebew Z, Nebiyu A, Zeleke N, Bogale S. 2011. Allelopathic effects of *Eucalyptus camaldulensis* Dehnh. on germination and growth of tomato. *American-Eurasian Journal of Agricultural and Environmental Sciences* 11: 600–608.
Forsyth GG, Richardson DM, Brown PJ, van Wilgen BW. 2004. A rapid assessment of the invasive status of *Eucalyptus* species in two South African provinces. *South African Journal of Science* 100: 75–77.
Gabriel WJ. 1975. Allelopathic effects of black walnut on white birches. *Journal of Forestry* 73: 234–237.
Galatowitsch S, Richardson DM. 2005. Riparian scrub recovery after clearing of invasive alien trees in headwater streams of the Western Cape, South Africa. *Biological Conservation* 122: 509–521.
Geldenhuys CJ. 2008. Practical guidelines for the rehabilitation of forest-related streambank vegetation with removal of invader plant stands along the Berg River, Western Cape. Working for Water Report no. FW - 02/08. Pretoria: Forestwood.
Gniazowska A, Bogatek R. 2005. Allelopathic interactions between plants. Multi-site action of allelochemicals. *Acta Physiologiae Plantarum* 27: 395–407.
Grbović S, Orčić D, Couladis M, Jovin E, Bugarin D, Balog K, Mimica-Dukić N. 2010. Variation of essential oil composition of *Eucalyptus camaldulensis* (Myrtaceae) from the Montenegro coastline. *Apteff* 41: 151–158.
Grman E, Suding KN. 2010. Within-year soil legacies contribute to strong priority effects of exotics on native California grassland communities. *Restoration Ecology* 18: 664–670.
Haddadchi G, Massoodi Khorasani F. 2006. Allelopathic effects of aqueous extracts of *Sinapis arvensis* on growth and related physiological and biochemical responses of *Brassica napus*. *Journal of Science, University of Tehran* 32: 23–28.
Hierro JL, Callaway RM. 2003. Allelopathy and exotic plant invasion. *Plant and Soil* 256: 29–39.
Holmes PM, Esler KJ, Richardson DM, Witkowski ETF. 2008. Guidelines for improved management of riparian zones invaded by alien plants in South Africa. *South African Journal of Botany* 74: 538–552.
Inderjit, del Moral R. 1997. Is separating resource competition from allelopathy realistic? *Botanical Review* 63: 221–230.
Inderjit. 2001. Soils: environmental effect on allelochemicals activity. *Agronomy Journal* 93: 79–84.
Inderjit, Wardle DA, Karban R, Callaway RM. 2011. The ecosystem and evolutionary contexts of allelopathy. *Trends in Ecology and Evolution* 26: 655–662.
Jairus T, Mpumba R, Chinoya S, Tedersoo L. 2011. Invasion potential and host shifts of Australian and African ectomycorrhizal fungi in mixed eucalypt plantations. *New Phytologist* 192: 179–187.
Jensen LB, Courtois B, Shen L, Li Z, Olofsdotter M, Mauleon RP. 2001. Locating genes controlling allelopathic effects against barnyard grass in upland rice. *Agronomy Journal* 93: 21–26.
Jose S, Williams R, Zamora D. 2006. Belowground ecological interactions in mixed-species forest plantations. *Forest Ecology and Management* 233: 231–239.
Khan MA, Hussain I, Khan EA. 2008. Allelopathic effects of Eucalyptus (*Eucalyptus camaldulensis* L.) on germination and seedling growth of Wheat (*Triticum aestivum* L.). *Pakistan Journal of Weed Science Research* 14: 9–18.
May FE, Ash JE. 1990. An assessment of the allelopathic potential of *Eucalyptus*. *Australian Journal of Botany* 38: 245–254.
Milchunas DG, Vandever MW, Ball LO, Hyberg S. 2011. Allelopathic cover crop prior to seeding is more important than subsequent grazing/mowing in grassland establishment. *Rangeland Ecology and Management* 64: 291–300.
Mohamadi N, Rajaie P. 2009. Effects of aqueous eucalyptus (*E. camaldulensis* Labill) extracts on seed germination, seedling growth and physiological responses of *Phaseolus vulgaris* and *Sorghum bicolor*. *Research Journal of Biological Sciences* 4: 1292–1296.
Mucina L, Rutherford MC. 2006. *The vegetation of South Africa, Lesotho and Swaziland. Strelitzia* 19. Pretoria: South African National Biodiversity Institute.
Niakan M, Saberi K. 2009. Effects of *Eucalyptus* allelopathy on growth characters and antioxidant enzymes activity in Phalaris weeds. *Asian Journal of Plant Science* 8: 440–446.
Padhy BB, Patanaik PK, Tripathy AK. 2000. Allelopathic potential of *Eucalyptus* leaf litter leachates on germination and seedling growth of fingermillet. *Allelopathy Journal* 7: 69–78.
Razavi darbar S, Lakzian A. 2007. Evaluation of chemical and biological consequences of soil sterilization methods. *Caspian Journal of Environmental Sciences* 5: 87–91.
Richardson DM, van Wilgen BW. 2004. Invasive alien plants in South Africa: how well do we understand the ecological impacts? *South African Journal of Science* 100: 45–52.
Richardson DM, Holmes PM, Esler KJ, Galatowitsch SM, Stromberg JC, Kirkman SP, Pyšek P, Hobbs RJ. 2007. Riparian vegetation: degradation, alien plant invasions, and restoration prospects. *Diversity and Distributions* 13: 126–139.
Richardson DM, Gaertner M. 2013. Plant invasions as builders and shapers of novel ecosystems. In: Hobbs RJ, Higgs EC, Hall CM (eds), *Novel ecosystems: intervening in the new ecological world order*. Oxford: Wiley-Blackwell. pp 102–114.

Romagni JG, Allen SN, Dayan FE. 2000. Allelopathic effects of volatile cineoles on two weedy plant species. *Journal of Chemical Ecology* 26: 303–313.

Ruwanza S, Musil CF, Esler KJ. 2012. Sucrose application is ineffectual as a restoration aid in a transformed southern African lowland fynbos ecosystem. *South African Journal of Botany* 80: 1–8.

Ruwanza S, Gaertner M, Esler KJ, Richardson DM. 2013a. Both complete clearing and thinning of invasive trees lead to short-term recovery of native riparian vegetation in the Western Cape, South Africa. *Applied Vegetation Science* 16: 193–204.

Ruwanza S, Gaertner M, Esler KJ, Richardson DM. 2013b. The effectiveness of active and passive restoration on recovery of indigenous vegetation in riparian zones in the Western Cape, South Africa. *South African Journal of Botany* 88: 132–141.

Sahin Basak S, Candan F. 2010. Chemical composition and *in vitro* antioxidant and antidiabetic activities of *Eucalyptus camaldulensis* Dehnh. essential oil. *Journal of Iranian Chemical Society* 7: 216–226.

Sasikumar K, Vijayalakshmi C, Parthiban KT. 2001. Allelopathic effects of *Eucalyptus* on redgram (*Cajanus cajan* L.). *Journal of Tropical Agriculture* 39: 134–138.

Schumann AW, Little KM, Eccles NS. 1995. Suppression of seed germination and early seedling growth by plantation harvest residues. *South African Journal of Plant and Soil* 12: 170–172.

Siemens TJ, Blossey B. 2007. An evaluation of mechanisms preventing growth and survival of two native species in invasive Bohemian knotweed (*Fallopia* ×*bohemica*, Polygonaceae). *American Journal of Botany* 94: 776–783.

Singh NB, Ranjana S. 2003. Effect of leaf leachate of *Eucalyptus* on germination, growth and metabolism of green gram, black gram and peanut. *Allelopathy Journal* 11: 43–52.

Singh HP, Batish DR, Setia N, Kohli RK. 2005. Herbicidal activity of volatile oils from *Eucalyptus citriodora* against *Parthenium hysterophorus*. *Annals of Applied Biology* 146: 89–94.

Southam CM, Erlich J. 1943. Effects of extract of western red-cedar heartwood on certain wood-decaying fungi in culture. *Phytopathology* 33: 517–524.

Souto XC, Bolaño JC, González L, Reigosa MJ. 2001. Allelopathic effects of tree species on some soil microbial populations and herbaceous plants. *Biologia Plantarum* 44: 269–275.

Sugiyama S, Yoshino T, Kanahara H, Shichiri M, Fukushi D, Ohtani T. 2004. Effects of acetic acid treatment on plant chromosome structures analyzed by atomic force microscopy. *Analytical Biochemistry* 324: 39–44.

Tererai F. 2012. The effects of invasive trees in riparian zones and implications for management and restoration: insights from *Eucalyptus invasions* in South Africa. PhD thesis, Stellenbosch University, South Africa.

Tererai F, Gaertner M, Jacobs SM, Richardson DM. 2013. *Eucalyptus* invasions in riparian forests: effects on native vegetation community diversity, stand structure and composition. *Forest Ecology and Management* 297: 84–93.

Tripathi S, Tripathi A, Kori DC. 1999. Allelopathic evaluation of *Tectona grandis* leaf, root and soil aqueous extracts on soyabean. *Indian Journal of Forestry* 22: 366–374.

Turpie JK, Marais C, Blignaut JN. 2008. The working for water programme: evolution of a payments for ecosystem services mechanism that addresses both poverty and ecosystem service delivery in South Africa. *Ecological Economics* 65: 788–798.

Valladares F, Sanchez-Gomez D, Zavala MA. 2006. Quantitative estimation of phenotypic plasticity: bridging the gap between the evolutionary concept and its ecological applications. *Journal of Ecology* 94: 1103–1116.

van Wilgen BW, Le Maitre DC, Cowling RM. 1998. Ecosystem services, efficiency, sustainability and equity: South Africa's Working for Water programme. *Trends in Ecology and Evolution* 13: 378.

Willis RJ. 1985. The historical base of the concept of allelopathy. *Journal of the History of Biology* 18: 71–102.

Zahed N, Hosni K, Brahim NB, Kallel M, Sebei H. 2010. Allelopathic effect of *Schinus molle* essential oils on wheat germination. *Acta Physiologiae Plantarum* 32: 1221–1227.

Zhang C, Fu S. 2009. Allelopathic effects of eucalyptus and the establishment of mixed stands of *Eucalyptus* and native species. *Forest Ecology and Management* 258: 1391–1396.

Zhang D, Zhang J, Yang W, Wu F. 2010. Potential allelopathic effect of *Eucalyptus grandis* across a range of plantation ages. *Ecological Restoration* 25: 13–23.

Received 9 June 2013, revised 8 May 2014, accepted 22 June 2014

Appendix 1: Generalised linear model ANOVA results for the effect of *Eucalyptus camaldulensis* aqueous extracts and soil treatments collected from the Berg River in the Western Cape province, South Africa, on growth parameters (germination [$\log_{10}$], shoot height, root length and total dry biomass [$\log_{10}$]) of four riparian plant species (*Vachellia karroo*, *Diospyros glabra*, *Dimorphotheca pluvialis* and *Olea europaea*) in a greenhouse based trial. Columns with different superscript letters and values in bold are significantly different at $P < 0.05$. Native+L = native + litter, Steril+L = sterilised + litter

Species	Aqueous treatment				Soil treatment					ANOVA $F_{(3;242)}$	ANOVA $F_{(4;242)}$	ANOVA $F_{(12;242)}$
	Tap	Leaf	Bark	Root	Native	Sterilised	Stand	Native+L	Steril+L	Aqueous treatment	Soil treatment	Aqueous × soil treatments
Germination (%)												
Vachellia karroo	28.79^{a}	26.88^{a}	29.07^{a}	27.58^{a}	44.83^{a}	27.27^{c}	36.01^{b}	18.62^{d}	20.56^{d}	0.36ns	**15.64**	0.74ns
Diospyros glabra	61.91^{a}	74.35^{a}	65.25^{a}	65.62^{a}	76.82^{ab}	86.17^{a}	79.22^{ab}	46.12^{c}	54.29^{c}	0.59ns	**5.73**	1.26ns
Dimorphotheca pluvialis	28.19^{a}	23.45^{a}	17.97^{ab}	14.80^{b}	51.49^{a}	8.92^{b}	65.83^{a}	9.63^{b}	12.36^{b}	**3.12**	**15.66**	1.35ns
Olea europaea	35.67^{a}	12.27^{bc}	10.22^{c}	23.60^{b}	25.82^{b}	35.32^{b}	60.88^{a}	5.11^{c}	6.94^{c}	**8.63**	**22.78**	**3.16**
Shoot height (cm)												
Vachellia karroo	2.70^{a}	2.36^{a}	2.22^{a}	2.52^{a}	2.83^{a}	2.18^{ab}	2.15^{ab}	2.50^{a}	2.56^{a}	1.58ns	**2.67**	1.20ns
Diospyros glabra	3.70^{a}	3.08^{b}	3.02^{b}	3.10^{b}	3.91^{a}	3.08^{b}	3.22^{b}	2.98^{b}	2.91^{b}	**8.38**	**10.73**	0.65ns
Dimorphotheca pluvialis	22.21^{a}	16.18^{b}	17.24^{ab}	15.42^{b}	29.40^{a}	11.99^{c}	21.81^{b}	10.24^{c}	15.38^{bc}	**3.82**	**20.27**	0.91ns
Olea europaea	1.24^{a}	1.15^{a}	1.12^{a}	1.36^{a}	1.63^{a}	1.26^{ab}	1.56^{a}	0.89^{b}	0.76^{b}	0.70ns	**7.26**	1.29ns
Root length (cm)												
Vachellia karroo	10.00^{a}	8.76^{b}	9.25^{ab}	9.78^{a}	10.41^{a}	9.83^{ab}	9.43^{ab}	8.76^{b}	8.73^{b}	**4.93**	**6.51**	**3.10**
Diospyros glabra	12.05^{a}	11.38^{a}	11.39^{a}	11.41^{a}	12.85^{a}	11.81^{ab}	11.95^{ab}	10.78^{bc}	10.40^{c}	1.43ns	**10.00**	0.88ns
Dimorphotheca pluvialis	5.39^{a}	3.55^{b}	3.57^{b}	3.16^{b}	5.94^{a}	2.87^{b}	4.73^{a}	3.05^{b}	3.00^{b}	**10.19**	**15.13**	1.29ns
Olea europaea	4.88^{a}	3.12^{b}	3.82^{ab}	3.51^{ab}	5.80^{a}	3.46^{b}	6.06^{a}	1.73^{b}	2.11^{b}	**3.33**	**19.23**	**3.87**
Total dry biomass (g)												
Vachellia karroo	0.39^{a}	0.23^{b}	0.21^{b}	0.26^{b}	0.33^{a}	0.30^{a}	0.23^{b}	0.19^{b}	0.28a^{b}	**3.63**	**6.31**	**2.12**
Diospyros glabra	1.23^{a}	0.55^{b}	0.40^{b}	0.50^{b}	1.18^{a}	0.65^{b}	0.63^{b}	0.38^{b}	0.44^{b}	**30.53**	**19.60**	**2.28**
Dimorphotheca pluvialis	27.27^{a}	13.96^{b}	13.38^{b}	12.88^{b}	51.17^{a}	10.57^{b}	24.67^{b}	8.23^{b}	9.52^{b}	**6.03**	**8.87**	0.47ns
Olea europaea	0.16^{a}	0.22^{a}	0.15^{a}	0.11^{a}	0.23^{a}	0.08^{c}	0.13^{b}	0.20^{a}	0.20^{a}	1.57	**3.08**	1.19ns

ns = Non-significant

Appendix 2: Gas chromatograms of *Eucalyptus camaldulensis* fresh leaf, bark and root samples and aqueous extracts used to water native plants