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ORIGINAL PAPER

Identification of volatile compounds released by roots of an invasive plant, bitou bush (*Chrysanthemoides monilifera* spp. *rotundata*), and their inhibition of native seedling growth

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Abstract Allelopathy has been suggested as a mechanism promoting the monoculture formation of some invasive exotic plants. Previous studies have shown that hydrophobic extracts of the roots and soil of exotic bitou bush (*Chrysanthemoides monilifera* spp. *rotundata* (DC.) T. Norl.) inhibited the seedling growth of five Australian native plants, including the dominant acacia (*Acacia longifolia* var. *sophorae* (Labill.) F. Muell.). Based on this finding, we compared the hydrophobic root and soil chemical profiles of bitou bush and acacia to determine whether bitou bush roots release allelopathic compounds that are novel to the invaded system. We detected three compounds that were exclusive to the bitou bush root and soil, and seven compounds that were common to the bitou bush and acacia roots but only present in the bitou bush soil. The compounds unique to the bitou bush invaded soil were all sesqui- and diterpenes. Several of these compounds were found to inhibit the seedling growth of a native sedge, *Isolepis nodosa* (Rott.) R. Br. Of particular interest are the sesquiterpenes: β-maaliene, α-isocomene, β-isocomene, δ-cadinene, 5-hydroxycalamenene and 5-methoxycalamenene which were found in high concentrations in the bitou bush root and soil extracts and exhibited phytotoxic activity. Therefore, we present evidence to suggest that bitou bush exudes low molecular weight volatile compounds into the soil which inhibit native plant seedling growth. The reduced establishment of native plants via allelopathy is likely to create space and contribute to the invasion of bitou bush on the eastern Australian coast.

Keywords Allelopathy · Chemical interference competition · Roots · Soil · Terpenes · Volatile compounds

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Introduction

Plant roots release organic compounds into the rhizosphere via decomposition, root cell sloughing, mucilage secretion and compound exudation (see reviews in Einhellig 1995; Kuzyakov and Domanski 2000; Whipps 1990). Root derived compounds, or rhizodeposits, have the ability to regulate the soil microbial community and the soil chemical and physical properties, and to affect the growth of neighbouring plants species (Bertin et al. 2003; Walker et al. 2003). Additionally, the soil biotic and abiotic conditions also have the potential to determine the persistence and chemical transformation of

rhizodeposits (Cheng 1995; Inderjit 2001). Rhizodeposits move through the soil or enter the atmosphere at different rates depending on the properties of the compound and the soil environment (Cheng 1995). Furthermore, site and compound specific transformation of the rhizodeposits is likely to occur as they come into contact with microbes (Inderjit 2005) and other compounds such as Mn and Fe oxides, which are powerful catalysts known to polymerise phenolic compounds and form humic acids (Huang et al. 1999). However the exact fate of root derived compounds in the soil is not well understood (Walker et al. 2003).

In previous studies we have shown that the hydrophobic extracts (dichloromethane and acetone soluble) of bitou bush roots, leaves and soil, consistently inhibited the germination and seedling growth of a range of native plants (Ens unpublished data), including the native dominant of the system, acacia (Austin 1978; Weiss and Noble 1984b). This study was designed to identify the hydrophobic compounds that were likely to be responsible for the observed inhibition of seedling growth by the crude hydrophobic root and soil extracts. We compared the root and soil chemical profiles of the bitou bush and the dominant native acacia using Gas chromatography–Mass spectrometry to determine whether bitou bush alters the soil chemistry of the eastern Australian sand dunes and also conducted bioassays on groups of hydrophobic compounds to detect possible bitou bush allelochemicals.

Hydrophobic components of plant roots are of increasing interest as allelopathic (Barney et al. 2005; Kong et al. 2002; Lin et al. 2007) and antimicrobial agents (Whitfield et al. 1981). There is a paucity of information on the hydrophobic fraction of soils (Jordan et al. 1993; Lin et al. 2007). Hydrophobic, oily or waxy substances are likely to have a long residence time in soil, particularly soils with little humic matter such as in the sand dunes of our study where they tend to coat particles and form hydrophobic skins (Roberts and Carbon 1972). Additionally, some plant-derived hydrophobic compounds that form skins around sand particles induce water repellency (Franco et al. 2000; McGhie and Posner 1981) which facilitates residence time and can inhibit germination of seeds (Osborn et al. 1967). High molecular weight hydrophobic compounds, such as the long chain alkanes, tend to be recalcitrant and are only broken down by specialist microbes able to produce biosurfactants (Roper 2004). Hydrophobic waxes and oils are well known to prevent desiccation and play a role in chemical defense in leaves (see reviews in Bargel et al. 2006; Post-Beittenmiller 1996). Although there is a paucity of published evidence for the presence and function of plant root waxes and oils, similar defense and protection functions are also postulated, particularly for plants adapted to dry areas such as coastal sand dunes where root-water evaporation is more of a risk.

Allelopathy has been shown to facilitate the invasion of some exotic species into previously diverse systems (Hierro and Callaway 2003; Knight et al. 2007). Despite the seemingly logical explanation of allelopathy as a mechanism of exotic plant invasion, controversy over its ecological relevance has been debated in the literature particularly as a result of methodological ambiguities and limited ecological application (Mallik 2002; Wardle et al. 1998; Williamson 1990). However, several hundred allelochemicals released from plants and microbes are known to affect the function of other species (Einhellig 1995) and recent studies into the mode of action of allelochemicals have clearly demonstrated allelopathy (Field et al. 2006; Hierro and Callaway 2003; Mitchell et al. 2006). The deployment of bioassays to demonstrate allelopathy has been criticized in the literature (Weidenhamer 1996), however they do have certain advantages when designed to answer specific questions, particularly in exploratory studies of allelopathy potential. For example, bioassays can be used to identify the presence of phytotoxins in different plant parts (localization), the subsequent release into the soil (exudation), toxic concentrations of compounds and mixtures, susceptible species, seedling morphology effects, and physiological mechanisms of growth inhibition (Einhellig 2002; Inderjit and Nilsen 2003). For parameters influencing the interpretation of allelopathy bioassays see Inderjit and Nilsen (2003).

The identification of phytotoxic chemicals in both the root and rhizosphere of an exotic plant species which are absent in the root and rhizosphere of dominant native plant systems may be suggestive of allelopathy. Demonstration of the root-soil allelochemical continuum is proposed as a valuable preliminary investigation into the likelihood of allelopathy. The present study followed this approach to

explore allelopathy as a mechanism of exotic plant invasion, which falls in the context of soil chemical ecology as suggested by Inderjit and Weiner (2001). We used the exotic bitou bush (*Chrysanthemoides monilifera* spp. *rotundata* (DC.) T. Norl.) invasion of the eastern Australian coast as a case study.

Exotic species

Bitou bush is a South African shrub in the Asteraceae family which was extensively planted on the sand dunes of the New South Wales coast of Australia from 1948 to 1964 to stabilize the sand dunes, particularly following sand mining (Agriculture and Resource Management Council of Australia & New Zealand et al. 2000; Weiss 1986). However, by 2000, bitou bush had invaded ~80% of the New South Wales coastline, including un-mined areas, and formed monocultures if left unmanaged (Agriculture and Resource Management Council of Australia & New Zealand et al. 2000; Weiss et al. 1998). In 2004, 96 plant populations and communities were declared threatened by bitou bush (DEC 2004). Previous research suggested that bitou bush displaced native plants through germination inhibition (Weiss et al. 1998), reduced native plant species richness and significantly altered the vegetation composition of dune communities (Brewer and Whelan 2003; Mason and French 2007). Past studies also suggested that bitou bush invasion may be facilitated by allelopathy. Bitou bush litter was found to significantly reduce the germination success of the native dominant species in this system, *Acacia longifolia* var. *sophorae* (Labill.) F. Muell) (Vranjic et al. 2000), cress (*Lepidium sativum*) and *Hardenbergia comptoniana* (Hughes 1998). The root and shoot biomass and median *Rhizobium* population of *A. sophorae* were also significantly lower when grown in bitou bush soil rather than *Acacia longifolia* var. *sophorae* soil (Vranjic et al. 2000). Aqueous leaf litter leachates of bitou bush were found to marginally affect the germination of *Eucalyptus viminalis*, *Allocasuarina littoralis* and *Hakea dactyloides* (Copeland 1984) and macerated bitou bush leaf solutions appeared to affect cress and *Schoenia filifolia* (Hughes 1998). Collectively, these studies indicate the possibility of allelopathy as a mechanism of bitou bush invasion, however further investigation is warranted.

Methods and materials

Root collection and extraction

Bitou bush roots (498.0 g) and acacia roots (499.7 g) were collected from at least five plants on the coastal sand dunes near Wollongong, New South Wales, Australia during June 2004. Voucher specimens are deposited at the Janet Cosh Herbarium, University of Wollongong: (*Chrysanthemoides monilifera* spp. *rotundata*) (9872-WOLL) and *Acacia sophorae* var. *longifolia* (9871-WOLL). The bitou bush and acacia roots were treated separately. They were gently washed with distilled water, manually chopped finely and soaked in dichloromethane (DCM) (HPLC grade) (1 l) for 30 h with intermittent agitation. After soaking, the liquid was removed by filtration and the DCM evaporated under reduced pressure (Büchi rotary evaporator) from a water bath (38°C) which produced crude brown resinous extracts (Stage 1 fractionation).

Soil collection and extraction

Soil from below the canopy of at least five bitou bush plants (soil mass 7,220 g) and five acacia plants (soil mass 5,980 g) was collected and pooled. Soil was collected from depths of 10–20 cm below the surface and within 10 cm of the live, visible roots. Particles less than 2 mm were sifted (2 mm aperture sieve, Endecotts Ltd, London, England) and used for analysis. DCM (2.5 l) was added to each of the pooled bitou bush and acacia soil samples and the hydrophobic fraction was extracted in the same manner as the roots.

GC–MS analysis of organic extracts

Samples of the four extracts from the bitou bush and acacia root and soil were re-dissolved in DCM (1 ml) and 0.5 µl injected into a HP 58980 gas chromatograph (GC) coupled to a VG Autospec mass spectrometer system (GC–MS). The GC–MS was fitted with a fused silica BP5 capillary column (30 m × 0.25 mm) (SGE Australia) operated in the split mode (20:1) with helium as the carrier gas. The oven temperature program began at 80°C, was increased by 4°C/min until 100°C, then increased by 10°C/min to 280°C and held at 280°C for 10 min.

The compounds were subsequently identified by comparison with mass spectra and Kovats retention indices published in the electronic NIST (2002) and Palisade (2004) libraries, and in Adams (2001).

Column chromatography fractionation of bitou bush root DCM extract

Column chromatography with silica gel 60 (0.040–0.063 mm; E. Merck) (10 g) was used to further fractionate the hydrophobic bitou bush root extract (0.512 g) with 3:7 (v/v) Petroleum spirit (HPLC grade, b.p. 40–60°C): DCM (HPLC grade) (200 ml) as the eluant. Twenty five aliquots of between 5 and 10 ml were collected from the column and seven main fractions were identified using thin layer chromatography (TLC) (Al-backed sheets; Merck Silica Gel 60 F_{254} with a fluorescent indicator) with DCM as the mobile phase and UV light ($\lambda =$ 254 nm) and iodine vapour for compound detection. These seven column fractions were subjected to GC and volatile component compounds were ascertained by comparison with previous GC–MS analyses of the bitou bush roots. Each fraction was also bioassayed for their effect on seed germination and seedling growth.

Bioassay of fractions: seed germination and seedling growth

To assess bioactivity of the fractions we adopted the dose response procedure with germination and seedling growth of native sedge, *Isolepis nodosa* (Rott.) R. Br., as indicators of plant response. *I. nodosa* was used based on its high germination success rate, susceptibility to bitou bush extracts as determined in previous studies (Ens unpublished data) and lower abundance in bitou bush invaded areas (personal observation). Seeds were collected from within the Wollongong area. Four replicates in Petri dishes of each of four concentrations (10, 100, 500, 1,000 ppm) for each fraction were prepared. Concentrations were based on the weight of the hydrophobic (DCM soluble) mixtures extracted from the bitou bush and acacia soil which were in the range of 200–900 ppm. Each fraction concentration was dissolved in DCM (1 ml) and the solution added to a glass Petri dish (9 cm diameter) fitted with Whatman No. 1 filter paper. The Petri dish was left in a fume cupboard for 20 min to ensure evaporation of the DCM and retention of the extract on the filter paper. Distilled water (2 ml) was added to each Petri dish and the pH recorded using an electronic pH meter (Activon model 209) after half an hour. Twenty *Isolepis nodosa* seeds were equidistantly placed in each Petri dish using a 1 cm grid. The Petri dishes were sealed with Parafilm® and incubated in a diurnal temperature and light regime of 15°C/25°C. Germination and root and shoot length after 23 days were recorded.

Statistical analyses

The germination, shoot length and root lengths as percentages of the controls were analysed separately by a 2-way ANOVA with fraction and concentration as fixed factors (SPSS Version 12.0). The Student–Neumann–Keuls (SNK) test was conducted to test differences among fractions and concentrations. The pH of each concentration was compared separately for each fraction using linear regression (SPSS Version 12.0).

Results

The crude hydrophobic extract of bitou bush roots (4.11 g) and soil (2.67 g) equated to 0.83% and 0.04% of the raw materials. Much less of the components of the acacia roots (0.5 g; 0.1%) and soil (2.06 g; 0.03%) were extractable in DCM. Subsequent GC–MS analysis revealed that the extracts consisted primarily of mono-, sesqui- and diterpenes, phenolic compounds, alkenes and alkanes (Fig. 1; Tables 1 and 2). The hydrophobic extract of the bitou bush root contained higher concentrations of alkenes, phenols and terpenes compared with the acacia root extract which contained primarily alkanes (41.2%) (Table 1). Of the compounds detected in the bitou bush soil, only the hexadecanol derivative was unique, while three unique compounds were also found in the bitou bush root (β-isocomene, 7-epi-silphiperfol-5-ene and manool) (Table 2). Six compounds were common to the bitou bush root and soil and acacia root, however they were absent from the acacia soil: β-maaliene, α-isocomene, δ-cadinene, 5-methoxycalamenene, 5-hydroxycalamenene, and the phenanthrenetriol derivative, 2-ethenyldodecahydro-2,

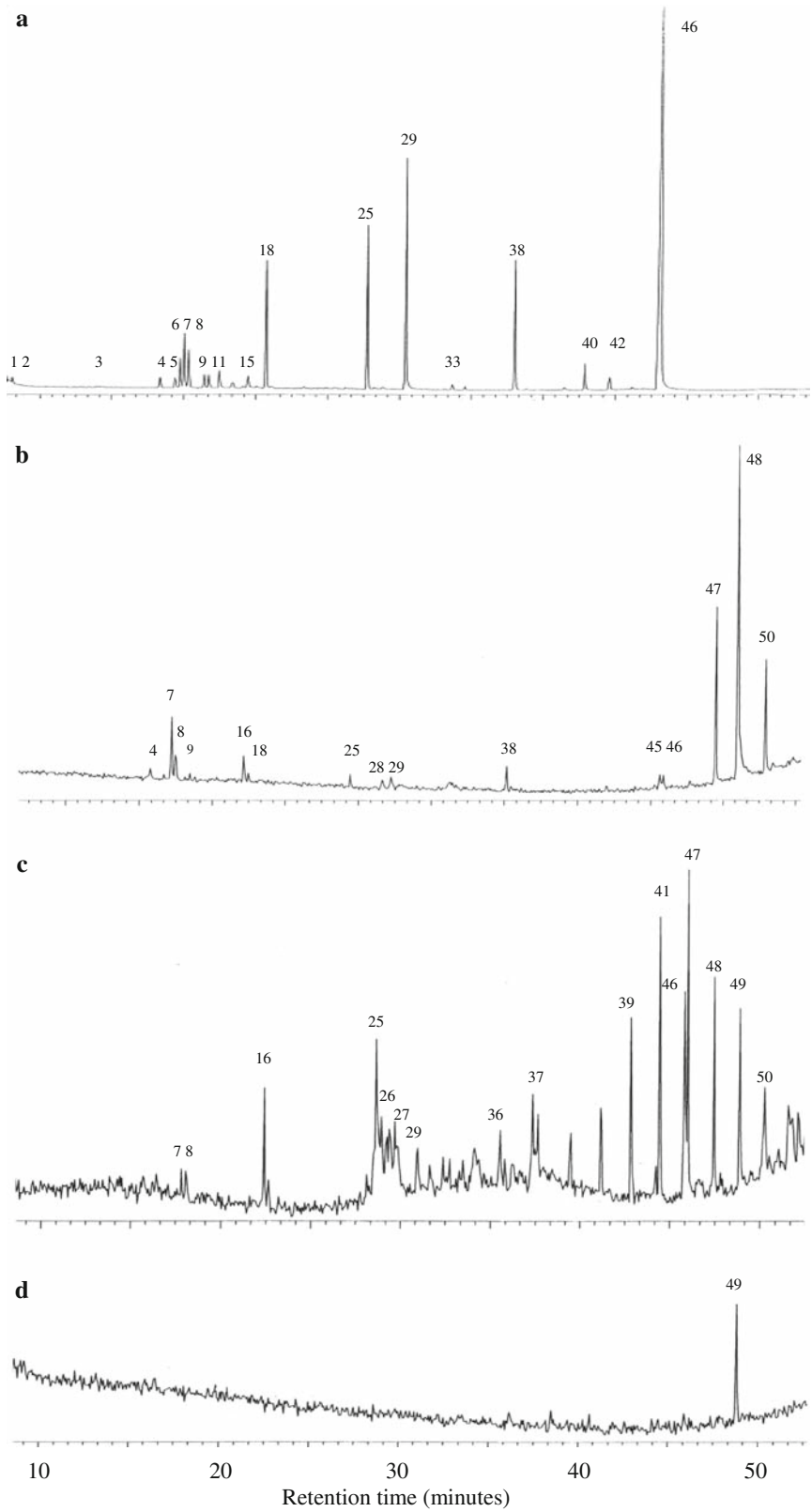


Fig. 1 Gas chromatograms of the hydrophobic extracts of (**a**) bitou bush root (**b**) bitou bush soil (**c**) acacia root and (**d**) acacia soil. Numbered peaks are annotated in Table 2

Table 1 The number and relative percent contribution of compounds in different chemical functional groups in the bitou bush and acacia root and soil hydrophobic extracts

Functional group	Number of functional group compounds (RA%) in each hydrophobic extract			
	Bitou bush root	Bitou bush soil	Acacia root	Acacia soil
Alkanes	–	3 (26.54)	15 (41.20)	1
Alkenes	1 (1.78)	–	–	–
Phenols	–	2 (4.09)	3 (9.74)	–
Sterols	1 (10.27)	1 (2.27)	–	–
Hydroxy terpenoids	8 (52.27)	4 (8.49)	3 (11.11)	–
Monoterpenes	3 (0.75)	–	–	–
Sesquiterpenes	21 (44.74)	7 (16.50)	5 (11.41)	–
Diterpenes	6 (42.26)	2 (4.44)	2 (8.45)	–

4b,8,8-tetramethyl-3,4,10a(1*H*)-phenanthrenetriol, 3-acetate (Table 2). Nine compounds were unique to the acacia root extract (Table 2), however none of these were detected in the acacia soil. Only an alkane was identified in the hydrophobic extract of the acacia soil. The relative area (%) of the GC volatile compounds of the hydrophobic extract of the bitou bush roots and soil and acacia roots are presented in Table 2.

Column chromatography fractionation of bitou bush root hydrophobic extract

Fraction 7 from the column chromatography separation on silica gel of the DCM extract contained two compounds and constituted the highest proportion of the bitou bush hydrophobic extract by weight, followed by fractions 2 and 1, which both contained numerous compounds on the basis of GC–MS analysis (Tables 3 and 4).

Fraction 1 contained 15 compounds with the major components being pentadecene (41.09%), 5-methoxycalamenene (**26** in Fig. 2) (20.34%) and pimaradiene (12.18%) (Table 4). Fraction 2 contained 20 compounds, with major components including 5-methoxycalamenene (**26** in Fig. 2) (46.12%), manool (22.90%) and δ-cadinene (8.10%) (Table 4). Fraction 3 largely contained manool (92.04%). Fractions 4 and 5 both contained primarily 5-hydroxycalamenene (**30** in Fig. 2) (74.80% and 86.08% respectively) and abietol (23.58% and 8.03% respectively) (Table 4). Fraction 7 contained an unidentified sterol and the phenanthrenetriol derivative (**46** in Fig. 2) (Table 4).

We were unable to isolate the pure compounds of fractions 4 and 7 by further column chromatography or preparative TLC for NMR spectroscopic analysis.

Bioassay of bitou bush root column fractions

Germination was not inhibited by any of the column fractions as the mean germination was always greater than 100% of the controls (Fig. 3). In fact, *I. nodosa* seed germination appeared to be stimulated particularly by fractions 1, 3, and 6 which were significantly higher than fractions 2, 4, and 7 (Table 5; Fig. 3).

The root and shoot length of *I. nodosa* were differentially affected by different column fractions at different concentrations (Table 5). For some fractions we did not obtain enough material to conduct bioassay assessments on the full range of concentrations (10–1,000 ppm) (Fig. 3a, e, f). Fraction 4 inhibited shoot length the most, followed by fractions 1 and 2 (significantly similar; $P < 0.05$), then fractions 3, 5, 6 (significantly similar; $P < 0.05$). Fraction 7 was not inhibitory (Fig. 3). At 500 ppm, fractions 4, 1 and 2 reduced *I. nodosa* shoot length to $\sim$30%, 50% and 60% (respectively) of the water control (Fig. 3). Similar patterns were found for the effect of each fraction on *I. nodosa* root length: application of fractions 1, 2 and 4 resulted in a 50% reduction of *I. nodosa* root length (Fig. 3). The *I. nodosa* roots and shoot lengths were significantly more affected by the higher concentrations (500 and 1,000 ppm) compared to the lower concentrations, suggesting an inhibition threshold at 500 ppm. There were no significant differences in the pH at each concentration for each fraction (Table 6).

Therefore, the primarily low molecular weight GC-volatile terpenes of fractions 1 and 2 and the phenolic compounds contained in fraction 4 at 500 ppm appeared to be most inhibitory to the growth of *I. nodosa*.

Table 2 Components of the bitou bush and acacia root and soil hydrophobic extracts

No.	Compound	MW[a]	RT[b]	RI[c]	KI[d]	RA[e] (%) of hydrophobic extract components[f]		
						Bitou bush root	Bitou bush soil	Acacia root
1	3-Carene	136				0.2		
2	3-Methoxy-*p*-cymene	164	16.45	1210	1235	0.2	–	–
3	2-Methoxy-*p*-cymol	164	16.63	1215	1245	0.3	–	–
4	Carvacrol ethyl ether	178	19.83	1309	1298	0.3	–	–
5	7-Epi-silphiperfol-5-ene	204	20.95	1342	1348	1.0	1.0	–
6	(+)-Cyclosativene	204	21.76	1366	1371	1.2	–	–
7	α-Copaene	204	22.00	1373	1377	2.6	–	–
8	*β*-Maaliene	204	22.15	1378	1382	3.9	5.8	0.6
9	α-Isocomene	204	22.35	1384	1388	3.1	3.8	0.7
10	*β*-Isocomene	204	22.85	1403	1407	1.7	0.9	–
11	Iso-caryophyllene	204	22.95	1405	1409	1.1	–	–
12	Cymene	194	23.36	1415	1427	1.1	–	–
13	α-Caryophyllene	204	24.42	1447	1455	0.4	–	–
14	Allo-aromadendrene	204	24.65	1463	1460	1.0	–	–
15	γ-Muurolene	204	25.18	1468	1480	0.3	–	–
16	Pentadecene	210	25.77	1492		1.8	–	–
17	Butylated hydroxytoluene	220	26.01		1516	–	2.6	3.4
18	α-Muurolene	204	26.28	1505	1500	0.4	–	–
19	*δ*-Cadinene	204	26.51	1508	1523	5.9	0.9	0.7
20	Cadala-1(10)3,8-triene	204	26.91	1526		0.6	–	–
21	α-Calacorene	200	27.60	1548	1546	0.6	–	–
22	Caryophyllene oxide	220	28.31	1571	1583	1.1	–	–
23	1,1,3-Trimethyl-3-phenylindane	236	28.70			–	–	2.2
24	Epi-α-muurolol	222	29.91	1624	1642	0.6	–	–
25	Calamenol	218	30.15	1632	1661	0.8	–	–
26	5-Methoxycalamenene (**26**)	232	32.54	1715		7.7	2.1	4.7
27	a Phenol	220	29.45			–	–	3.7
28	a Phenol	220	29.54			–	–	2.7
29	Hexadecanol derivative	296	32.124			–	1.5	–
30	5-Hydroxycalamenene (**30**)	218	34.26	1776		9.6	2.0	2.5
31	2,3,5,6-Tetrahydro-3,3,5,5-tetramethyl-*s*-indacene-1,7-dione	242	36.37			–	–	2.3
32	$C_{19}H_{40}$		36.56		1900	–	–	0.5
33	$C_{20}H_{42}$		38.48			–	–	0.8
34	Pimaradiene	272	39.07	1930	1950	0.8	–	3.3
35	$C_{21}H_{42}$		39.25			–	–	1.2
36	Sandaracopimaradiene	272	39.78	1944	1969	0.5	–	–
37	$C_{22}H_{44}$		40.33			–	–	2.4
38	$C_{23}H_{46}$		42.03			–	–	3.3
39	Manool	290	42.19	2113	1965	7.4	2.4	–
40	$C_{24}H_{48}$		44.22			–	–	4.4
41	Abietol	288	47.11	2300		2.7	–	–
42	$C_{25}H_{52}$	352	47.28			–	–	5.5

Table 2 continued

No.	Compound	MW[a]	RT[b]	RI[c]	KI[d]	RA[e] (%) of hydrophobic extract components[f]		
						Bitou bush root	Bitou bush soil	Acacia root
43	Abietol	288	49.25	2402	2402	1.8	–	–
44	Branched alkane		51.36			–	0.9	–
45	Unknown sterol		52.58	2555		10.3	2.3	–
46	2-Ethenyldodecahydro-2,4b,8,8-tetramethyl-3,4,10a(1*H*)-phenanthrenetriol ,3-acetate (**46**)	364	52.63	2566		29.1	2.0	5.2
47	Branched alkane		53.85			–	–	–
48	$C_{26}H_{54}$	366	54.30			–	15.1	7.3
49	Unknown		56.15				46.3	
50	$C_{27}H_{56}$	380	56.30			–	–	5.1
51	$C_{28}H_{58}$	394	58.30			–	10.5	3.9
52	$C_{29}H_{60}$	408	60.30			–	–	3.2
53	$C_{30}H_{62}$	422	62.30			–	–	1.9

[a] MW, Molecular weight from GC–MS data

[b] RT, Experimental retention time (mins) determined on a BP5 column using a homologous series of *n*-alkanes

[c] RI, Experimental retention index

[d] KI, Kovats index: KI = 100 $(T_u - T_c)/(T_{c+1} - T_c)$ + 100C, where T_u is the retention time for the unknown, T_c and T_{c+1} are the retention times for the *n*-alkane standards which bracket the unknown and C is the number of carbon atoms in the *n*-alkane standard that elutes prior to the unknown

[e] RA, Relative peak area (peak area relative to total peak area)

[f] Chemical components of the acacia soil are not included as we only found one compound (see Fig. 1d)

Table 3 Weights and percentage weights of each column chromatography fraction obtained from the bitou bush root hydrophobic (DCM) extract

Column fraction	1	2	3	4	5	6	7	Total
Weight (mg)	74.8	78.3	35.5	41.1	40.9	13.6	218.9	503.1
% weight	14.6	15.3	6.9	8.0	8.0	2.7	42.7	98.2

Discussion

The chemical profile of the bitou bush invaded root-soil system was distinctly different from the native acacia root-soil system. Bitou bush roots and soil contained compounds that were not found in the native root and soil and the native root and soil system contained compounds not detected in the bitou bush invaded system. We have therefore shown that this exotic woody weed changes the soil chemistry of its new environment. Mixtures of the compounds unique to the bitou bush roots and soil were shown to inhibit the growth of a native sedge in this study. Hence we suggest that South African bitou bush is allelopathic in the Australian environment. This evidence complements our previous study (Ens unpublished data) which found that the seedling growth of several Australian native plants was inhibited by the hydrophobic extracts of the bitou bush root and soil.

In the present study, the hydrophobic extract of the bitou bush roots contained higher concentrations of alkenes, hydroxylated terpenoids and terpenes than the acacia root which primarily contained alkanes (C_{19}–C_{32} alkane series). Of note was the high level of sesqui- and di-terpenes found in the hydrophobic bitou bush root (87%) and soil (20.9%) extracts. Terpenes play a significant role in determining ecosystem composition and function (Langenheim 1994) and have been implicated in plant defense against vertebrates, invertebrates and microbes,

Table 4 GC–MS detection of compounds in each column fraction of the bitou bush root hydrophobic extract

Column fraction	Compound	RA (%)
1	β-Maaliene	2.66[a]
	α-Isocomene	1.97[a]
	β-Isocomene	0.90[a]
	Allo-aromadendrene	1.97
	γ-Muurolene	1.32
	Pentadecene	41.09
	α-Muurolene	2.69
	δ-Cadinene	1.25[a]
	Cadala-1(10)3,8 triene	5.37
	Caryophyllene oxide	1.43
	5-Methoxycalamenene (**26**)	20.34[a]
	Pimaradiene	12.18
	Sandaracopimaradiene	6.09
2	7-Epi-silphiperfol-5-ene	0.20[a]
	α-Copaene	1.31
	β-Maaliene	2.66[a]
	α-Isocomene	2.22[a]
	β-Isocomene	1.00[a]
	Cymene	4.20
	Allo-aromadendrene	1.00
	Pentadecene	4.19
	δ-Cadinene	8.10[a]
	5-Methoxycalamenene (**26**)	46.12[a]
	Pimaradiene	1.78
	Sandaracopimaradiene	1.14
	Manool	22.90[a]
3	5-Methoxycalamenene (**26**)	5.31[a]
	Sandaracopimaradiene	1.77
	Manool	92.04[a]
4	5-Hydroxycalmenene (**30**)	74.80[a]
	Abietol	23.58
5	Caryophyllene oxide	2.56
	5-Methoxycalamenene (**26**)	2.42[a]
	5-Hydroxycalamenene (**30**)	86.08[a]
	Abietol	8.30
6	a Sterol	8.85[a]
	Abietol	47.79
	a Sterol	43.36[a]
7	a Sterol	30.0[a]
	2-Ethenyldodecahydro-2, 4b, 8, 8-tetramethyl-3, 4, 10a(1*H*)-phenanthrenetriol , 3-acetate (**46**)	70.0[a]

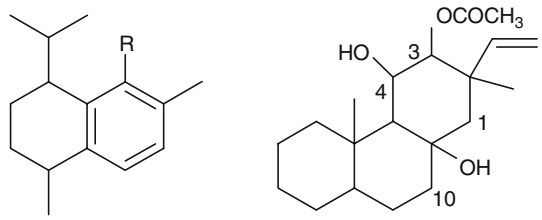
Compounds greater than 1% relative abundance (RA) are shown, except for those that were unique to the bitou bush invaded soil

[a] Denotes compounds that were detected in bitou bush soil

attraction of symbiotic organisms and pollinators, nutrient cycling (White 1994) and allelopathy Duke and Oliva 2004; Fischer et al. 1994; Raniello et al. 2007).

The chemical profile of the acacia root hydrophobic extract was characterized by the presence of the C_{19}–C_{32} alkane series. To our knowledge, this is the first documentation of an alkane series in a dicot root. Studies on monocot roots, particularly of pasture grasses, have shown that different species exhibit unique alkane series signatures (Roumet et al. 2006). Plant derived long chain alkanes have been shown to induce water repellency, particularly in sandy soils. Long chain root alkanes are likely to function as a root-soil barrier in older roots. Following root death they are likely to persist in sandy soils and bind to sand particles unless they are broken down by specialist bacteria (Roper 2004). The presence of long chain alkanes found in the acacia soil of this study suggests that the alkanes do persist in the soil and may have several functions in the native ecosystem. The acacia soil alkanes may facilitate essential symbiotic rhizobia and other bacteria (Roper 2004). Second, the production of alkanes may play a role in niche construction whereby the low soil water retention rates inhibit the germination of other plants in the vicinity of the acacia. If the alkanes serve as a carbon source for some microbes, the absence of root alkanes in bitou bush and the release of structurally different compounds may therefore alter the soil microbial community which may in turn alter floral composition (de Boer et al. 2006) and ecosystem function.

Six compounds were common to the bitou bush roots, acacia roots and bitou bush soil, however were not detected in the acacia soil. Furthermore, 10 of the bitou bush root compounds were detected in the bitou bush soil while only one acacia root compound, an alkane, was detected in the acacia soil. A number of explanations may account for the absence of compounds in the acacia soil. In line with the strategy to conserve nutrients deployed by acacia on the sand



30 R = OH
26 R = OCH_3

46

Fig. 2 Primary dichloromehane extractable constituents of the hydrophobic bitou bush root and soil extracts which demonstrated phytotoxic activity against *I. nodosa*. Compound numbers refer to those used to designate compounds (left hand column) in Table 2

dunes (Weiss and Noble 1984a), the acacia root may not exude many compounds, rather recycling them by resorbing and redistributing them prior to root death. Alternatively, the compounds may be released and subsequently transformed by the potentially different microbial community that is associated with the acacia. Finally, given that bitou bush has faster root growth and greater root biomass than coastal acacia (Weiss and Noble 1984a), there is likely to be an increased concentration of root exudates, root cell sloughing and root turnover (Iijima et al. 2003) and therefore a greater release of compounds into the soil, enabling better detection.

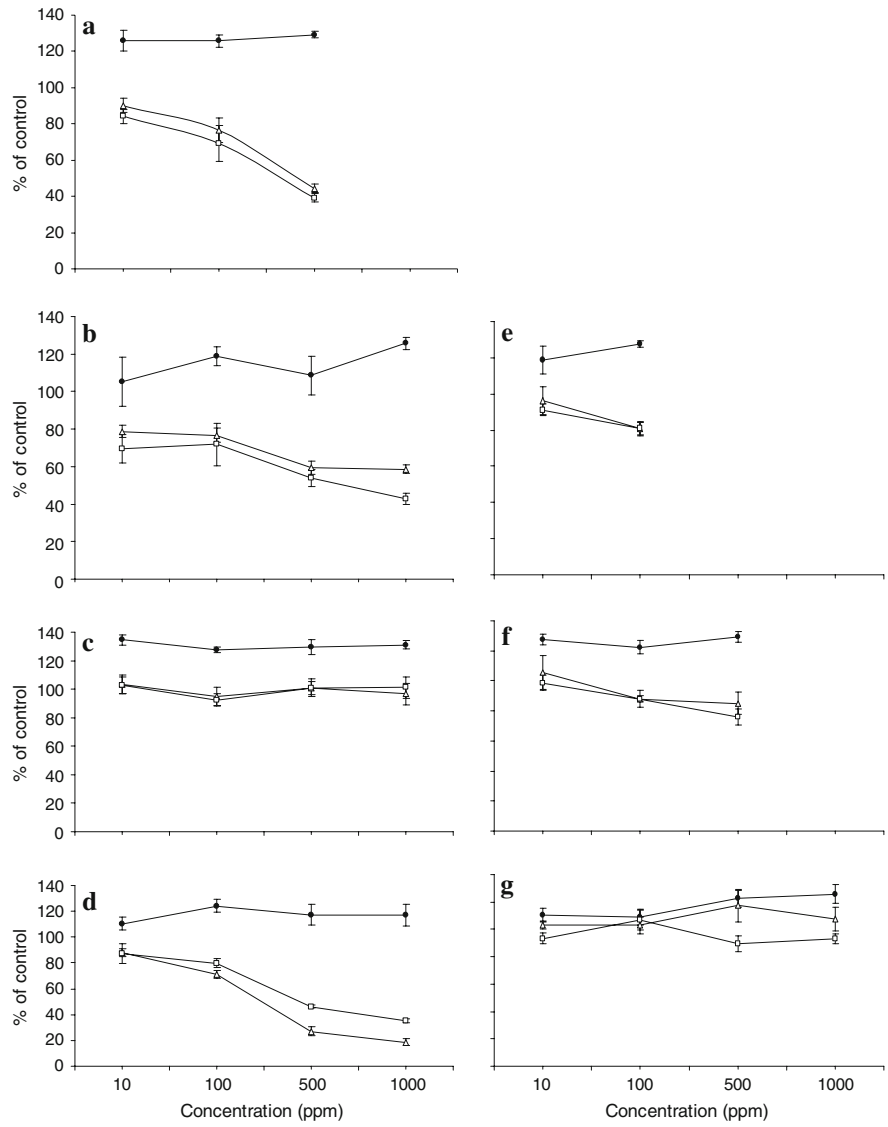


Fig. 3 Mean dose response curves of *I. nodosa* to each column fraction (**a**–**g**) of the hydrophobic bitou bush root extract. *Closed circles* indicate the germination response, *open triangles* the shoot length and *open squares* the root length expressed as a percentage of the control after 23 days of incubation. Error bars represent one standard erro

Table 5 Two-factor ANOVA results testing the effect of column fraction (CF) and concentration (C) on the germination and root and shoot lengths (as percentages of the control) of *I. nodosa* after 23 days of incubation

	df	Germination		Shoot length		Root length	
		F ratio	*P*	F ratio	*P*	F ratio	*P*
Column fraction (CF)	6	4.55	0.001	41.69	<0.001	34.88	<0.001
Concentration (C)	3	1.97	0.126	21.10	<0.001	24.48	<0.001
CF × C	14	0.99	0.477	6.07	<0.001	4.26	<0.001

Significance level: $P < 0.05$

Table 6 Regression results and mean pH (+standard errors) showing that there was no difference ($P > 0.05$) in the pH of increasing concentrations of each column fraction

	Column fractions						
	1	2	3	4	5	6	7
Number of different concentrations	3	4	4	4	2	3	4
Mean pH (+SE)	8.86 (0.03)	8.28 (0.16)	8.08 (0.19)	7.97 (0.09)	8.13 (0.12)	8.15 (0.23)	8.03 (0.05)
F ratio	12.57	3.21	0.16	0.01	*	1.44	0.68
P	0.18	0.22	0.73	0.93	*	0.44	0.50

*F ratio's were not calculated for column fraction five as the sample size was not greater than two

The root and shoot length of native sedge, *I. nodosa*, were significantly reduced by several column fractions (1, 2 and 4) of the bitou bush root hydrophobic extract. These fractions contained eight of the 10 compounds unique to the bitou bush root-soil with the most notable being the 5-hydroxycalamenene (**30**) which made up 74.8% of the GC-volatile components of fraction 4. Fraction 4 was also the most inhibitory fraction. This is the first report of the probable exudation of this compound and its phytotoxic, and therefore allelopathic, behaviour. Inhibition against the plant pathogenic fungi *Cladosporium cucumerinum* and *Pyricularia oryzae* was shown by 5-hydroxycalamenene which was isolated from the liverwort *Bazzania trilobata* (Scher et al. 2004). Antimicrobial activity of 5-hydroxycalamenene was also found as a function of the wound protection compounds exuded by *Tilia* spp. (Melcher et al. 2003). A related compound, 7-hydroxycalamenene, also isolated from *B. trilobata*, was shown to be inhibitory against *Phytophthora infestans*, *Botrytis cineraria*, *Septoria tritici*, *C. cucumerinum* and *P. oryzae* (Scher et al. 2004). Similarly, 5-methoxycalamenene (**26**) was a dominant component of the unique root-soil continuum found in the bitou bush system and also constituted 46% of fraction 2 and 20% of fraction 1 (based on the GC-volatile components) which were both significantly inhibitory towards *I. nodosa*. We have found no other documented evidence for the biological activity of this compound. The phenanthrenetriol derivative (**46**) constituted the greatest proportion of the hydrophobic bitou bush root extract and most of fraction 7, however we did not find that this fraction inhibited the growth of *I. nodosa*. This phenanthrenetriol derivative has also been documented as a dominant component of other *Chrysanthemoides* spp. roots (Bohlmann and Grenz 1979).

We therefore present preliminary evidence to suggest that bitou bush alters the soil chemistry of its new host environment by releasing different terpenes, and terpenes in general at a higher concentration, than the locally dominant native species. Mixtures of bitou bush root terpenes, that were also found in the surrounding soil, were shown to be phytotoxic against a native sedge in this study and may have antimicrobial activity as suggested by other researchers.

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