Bitou bush invasion is facilitated by soil chemistry changes which inhibit the growth of native plants

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Summary Bitou bush is a weed of national significance and has been declared as a key threatening process in NSW. We aimed to strengthen the scientific understanding of the mechanisms of invasion by investigating potential allelopathy and indirect soil chemical effects.

Our study compared whether extracts of bitou bush leaves, roots and soil had a different effect on the seedling growth of a range of native species compared to comparable extracts from an acacia, the native dominant in the non-invaded system. We found that bitou bush roots released significantly higher concentrations of sesquiterpenes into the soil, compared to the acacia. Corresponding bitou bush root and soil extracts were found through lab based bioassay studies to inhibit seedling growth of four native species. Moreover, acacia seedling growth was significantly inhibited only by the bitou bush soil extracts, suggesting an indirect soil chemical effect.

We also found that the acacia soil had higher concentrations of phenolic compounds than the bitou bush invaded soil. Several acacia root, leaf and soil extracts also inhibited the growth of native seedlings, although to a lesser extent than the bitou bush extracts. These results suggest that although there appeared to be allelopathic effects between co-evolved plants, the exotic bitou bush inhibited the seedling growth of all five native plant species studied, including the dominant acacia. This finding suggests that bitou bush dominance could at least partially be due to allelopathy or indirect soil effects.

Keywords Allelopathy, interference, competition, invasion mechanisms.

INTRODUCTION

Bitou bush (*Chrysanthemoides monilifera* spp. *rotundata* L.) is a South African shrub that was extensively planted on the New South Wales (NSW) coastal (and some inland) sand dunes following sand mining, particularly from 1946 to 1968 (DEC 2006). However by the 1980s bitou bush was noted as spreading into undisturbed tracts of native vegetation (Love 1984). In 1999 it was declared a Key Threatening Process in NSW and in 2000 declared a weed of national significance (DEC 2006).

Over the last 20 years, eight biological control agents have been released, and millions of dollars have been spent on chemical, mechanical and manual control (DEC 2006). However, bitou bush persists and continues to invade native vegetation and form monocultures if left unmanaged. In order to facilitate more effective management and use of public funds, further understanding of the invasion mechanisms and ecosystem impacts is required to inform best management and restoration practice.

There is preliminary evidence to suggest that allelopathy is a factor driving the invasion. Copeland (1984) and Hughes (1998) have both conducted rudimentary studies showing that several native species are affected by aqueous extracts of bitou bush. Vranjic *et al*. (2000) have also shown that bitou bush litter affected the establishment of acacia. To further our understanding of the interference competition between bitou bush and the native flora we devised a comprehensive bioassay protocol and complemented this study with a novel field based compound extraction method to determine whether allelopathy or indirect soil chemical effects were likely to inhibit the growth of several native plant species. We also attempted to identify the likely compounds.

MATERIALS AND METHODS

Bioassay procedure Four extracts using solvents of increasing hydrophobicity were prepared from the roots, leaves and soil of bitou bush and acacia (*Acacia longifolia* var. *sophorae* (Labill.) F.Muell) that were collected in Puckey's Estate, Wollongong, during July 2004. Leaves and roots were collected from five plants and the soil was collected from within 10 cm of plant roots. A range of solvents was used to try and identify the type of compounds that may be allelopathic. Increasing concentrations (0, 10, 50, 100, 500, 1000 ppm in distilled water) of these extracts were applied to Petri dishes. Twenty seeds were arranged equidistantly in each Petri dish and four replicates of each treatment (24 extracts and six concentrations) were

prepared for each of five native species: *A. longifolia* var. *sophorae* (A), *Lomandra longifolia* Labill. (L), *Isolepis nodosa* (Rottb.) R.Br. (I), *Actites megalocarpa* (Hook.f.) Lander (Ac), and *Banksia integrifolia* L.f. (B). Seeds were incubated for between 23 and 53 days depending on the species, in a diurnal (12 h/12 h) temperature (15°C/25°C) and light regime. If inhibition of seedling growth (root or shoot) was found for the leaf or root extracts only, phytotoxicity was suggested; if comparable (i.e. extracts derived from the same solvent) leaf or root *and* soil extracts inhibited seedling growth, then allelopathy was suggested; if only the soil extracts affected the seedling growth and not the comparable leaf or root extracts, indirect soil chemical effects were suggested. If the mean root or shoot length was less than 50% of the control, inhibition was cited. An ANOVA was also conducted to determine whether there was a significant effect of increasing concentration, and whether seedling growth was differently affected by the bitou bush and acacia extracts (statistics not provided in this paper).

Gas chromatography – mass spectrometry of hydrophobic root and soil extracts Samples of the hydrophobic bitou bush and acacia root and soil extracts were injected into a Varian 3700 gas chromatograph (GC) coupled to a VG Autospec mass spectrometer (MS) system (GC-MS). All compounds conducive to this method were identified by comparison with mass spectra and Kovats retention indices published in the electronic NIST library (2002) and Adams (2001).

Resin bag compound extraction *in situ* Calico bags filled with Amberlite XAD4® resin (10 g; Rohm Hass and Co.), which is designed to adsorb hydrophobic compounds, were prepared. Five bags were buried 10 cm below the soil surface at each of five locations at Corrimal Beach under each of three habitat types: bitou bush invaded, native vegetation and bare sand. After 10 days the bags were removed, soaked in dichloromethane (DCM) for 24 h and the extract dried under reduced pressure. The chemical constituents of the extract were identified using GC-MS as described above. Relative peak areas were calculated and the differences between each location and habitat were analysed using ANOVA (results not described here).

Table 1. Summary of phytotoxic, allelopathic and indirect soil effects of the bitou bush and acacia extracts on five native species: *A. longifolia* var. *sophorae* (A), *Lomandra longifolia* (L), *Isolepis nodosa* (I), *Actites megalocarpa* (Ac), and *Banksia integrifolia* (B).

Extract species	Plant part	Solvent extract	Type of effect		
			Phytotoxic	Allelopathic	Indirect soil effect
Acacia	leaf	DCM	L, A	I	
		acetone	L, I		
		methanol	L		
		water	L		
	root	DCM	A, Ac	I	
		acetone	B, I		
		methanol	Ac		
		water	A		
	soil	DCM			B, L
		acetone			L
		methanol			
		water			+ I
Bitou bush	leaf	DCM	A, L	I	
		acetone	I		
		methanol	Ac, L, I		
		water	A, L,		
	root	DCM	A, L	Ac, I	
		acetone	I	B	
		methanol	A, Ac, I		
		water	A, B	Ac, L	
	soil	DCM			B
		acetone			A
		methanol			
		water			+ I

RESULTS

The hydrophobic (DCM and acetone soluble) extracts of the bitou bush root and soil appeared to be more allelopathic than the other extracts (Table 1, E.J. Ens unpublished).

The hydrophobic bitou bush root (not shown here) and soil extracts were found to have significantly higher concentrations of a range of terpenes compared to the acacia soil and bare sand (Figure 1, statistics not shown here, Ens *et al.* 2008), which we suggest are responsible for the observed inhibition of native seedling growth.

DISCUSSION

Our extensive chemical comparisons of bitou bush invaded soil, native soils and bare *in situ* sand suggest that although all substrates contain many similar compounds, the bitou bush invaded sand is characterised by high concentrations of a range of sesquiterpenes and lacks the phenols found in acacia dominated native soil. The sesquiterpenes were also found in high concentrations in bitou bush roots and were absent from acacia roots. These results suggest that bitou bush is exuding these compounds into the sand and significantly altering the soil chemistry of invaded coastal dunes.

Furthermore, mixtures of bitou bush derived sesquiterpenes have been found to inhibit the growth of a range of native plant species. Considering both this hydrophobic (DCM and acetone soluble) component and the hydrophilic (methanol and water soluble) component of the bitou bush invaded system, it appears that although the acacia extracts have the potential to interfere with three native species (including the dominant acacia) bitou bush has the capacity to inhibit all five test species in the field via allelopathy or indirect soil effects.

Therefore, although we found evidence for interference competition between co-evolved plants, it appears that bitou bush is likely to become dominant by inhibiting the growth of many native species, and in particular, the native dominant acacia.

Amelioration of this invasion mechanism may be achieved by leaving a six month lag time following removal or mortality of the bitou bush to allow volatilisation of the low molecular weight, volatile terpenes (Komenda and Koppman 2002). Fire could also be used to speed up the volatilisation of the allelochemicals identified through our studies, by increasing soil temperatures. Additionally, established juvenile plants could be planted to facilitate restoration of the invaded system, as we suspect that more mature plants would not be as susceptible to the allelochemicals as the seedlings were found to be in our study.

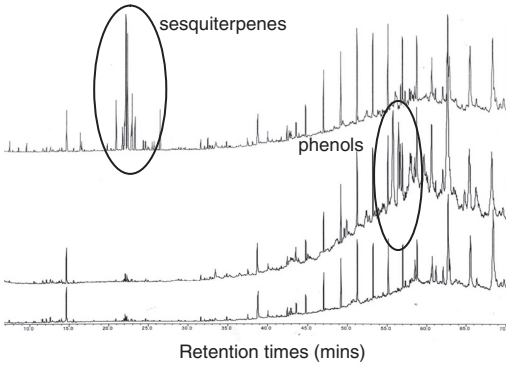

Figure 1. Gas chromatograms of the resin bag extracts from the bitou bush invaded (top), native vegetation (middle) and bare sand habitats (bottom). Adapted from Ens *et al.* (2008).

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