Indigenous plant recruitment limitation by bitou bush (Chrysanthemoides monilifera spp. rotundata): effect on life history stages and allelopathic mechanisms

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Indigenous plant recruitment limitation by bitou bush (*Chrysanthemoides monilifera* spp. *rotundata*): effect on life history stages and allelopathic mechanisms

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Abstract

Exotic plant invasion, the consequent displacement of indigenous flora and subsequent effects on ecosystem health has become of increasing concern to land managers, conservationists and government agencies. Despite the concomitant attention of ecologists and invasion biologists, our empirical understanding of the impacts and mechanisms of exotic plant invasion remains rudimentary and fragmented and further complicated by species and site specific effects. Exotic plant invasion is of paramount concern in Australia due to the high species endemism and the recent settlement of Europeans (in 1788) which has been paralleled by vast, rapid modification of the landscape. Large expanses of land have subsequently been cleared for agriculture, residential and industrial areas and many exotic species have been introduced, both intentionally and accidentally. As a result, exotic species invasion has become an issue of national significance.

In attempt to further our ecological understanding of the impacts, and macro and micro-mechanisms of exotic plant invasion, I have focused my research on the bitou bush (*Chrysanthemoides monilifera* spp. *rotundata* (DC.) T. Norl.) invasion of the eastern Australian coastal dune systems. Bitou bush has been declared Australia's sixth worst weed based on its invasibility and impacts on the environment. However there is a paucity of quantitative evidence to support these claims with substantiation being primarily anecdotal. Therefore I aimed to investigate the plant demographic impacts and soil chemistry changes imposed by the invasion and determined whether allelopathy and indirect soil chemical interference are mechanisms facilitating bitou bush invasion in Australia.

The demographic response of indigenous plants to the invasion of exotic woody plants has rarely been quantified. I therefore aimed to determine which life history stages of three indigenous plant species: *Correa alba* var. *alba* (Andrews; Rutaceae), *Monotoca elliptica* ((Sm.) R.Br.; Epacridaceae) and *Lomandra longifolia* (Labill.; Lomandraceae), were more susceptible to the invasion of bitou bush. I also assessed whether various morphological and physiological parameters of the mature stage of these species were affected by the presence of bitou bush. Populations of all three indigenous species in bitou bush invaded habitats had significantly fewer small individuals and a lower population density than populations in non-invaded habitats. The mean flower production, growth, ratio of reproductive: vegetative buds and physiological stress of mature individuals of each of these species in bitou bush invaded habitat did not differ from those in the non-invaded habitat. However, the flower production of *C. alba* was significantly more variable in the bitou bush invaded habitat which suggested plasticity in resource allocation in response to the invasion. Increased trait variability was not found for *M. elliptica* and *L. longifolia* suggesting mature plant tolerance to the new neighbour. We therefore propose that bitou bush affected indigenous plant populations primarily by preventing recruitment through the germination or seedling growth stages and that older plants typically tolerated the presence of the exotic. The reduction in indigenous plant recruitment is likely to create space that would facilitate bitou bush monoculture formation in the new host environment.

A more detailed assessment of the physiological health of mature indigenous plants in invaded habitats was conducted to determine whether there was seasonal effect of the invasion. The photosynthetic efficiency of plants was adopted as an indicator of physiological health. The seasonal photosynthetic patterns of *C. alba*, *M. elliptica* and *L. longifolia* in invaded and non-invaded habitats were assessed using chlorophyll

fluorescence. I also examined whether bitou bush altered the habitat physico-chemical parameters which may have lead to any observed changes in the physiological health of mature individuals. All three species exhibited photosynthetic maxima during winter and minima in summer, in contrast to most other Northern hemisphere studies on seasonal photosynthetic patterns. Winter photosynthetic maxima are likely to be facilitated by the autumn rains and cooler winter temperatures of the eastern Australian coast. Differences in the photosynthetic capacity of individuals of all three species among different sites were also detected. Although the invasion of bitou bush significantly altered the canopy cover of *C. alba* and *M. elliptica* and moderated the ground level microclimate, I detected no effect on the seasonal photosynthetic patterns of the three species studied, suggesting physiological tolerance to the invasion by mature plants. The reductions in ground incident light and daily maximum temperatures associated with the invasion were likely to be responsible for the reduction in variability of Fv/Fm (physiological stress parameter) detected in autumn for all species. Therefore, I suggest that the photosynthetic patterns of Australian native plants is a function of seasonal climatic and site variability, which was not significantly affected by the microhabitat changes induced by the invasion of bitou bush.

Chemical interference is increasingly suggested as a mechanism facilitating exotic plant invasion. I therefore devised a comprehensive bioassay technique that promoted detection and differentiation of phytotoxicty, allelopathy and indirect soil effects of exotic plants by comparing extract inhibition with that of a dominant indigenous plant. Comparison of the bioactivity of comparable extracts from plant parts and soil was integral to the technique. Hydrophilic to hydrophobic solvent extracts of indigenous acacia and exotic bitou bush leaves and roots all exhibited differential phytotoxic effects on a range of

indigenous plants. Chemical interference, or allelopathy, between co-evolved plants was found by the hydrophobic extracts of the roots and soil of acacia against a sedge, *Isolepis nodosa* (Rott.) R. Br. Hydrophobic and hydrophilic extracts of the roots and soil from the exotic bitou bush elicited allelopathic effects against four indigenous species. Additionally, the hydrophobic soil extracts of bitou bush inhibited the germination and growth of *Banksia integrifolia* and *A. longifolia* var. *sophorae*, while the acacia soil extract inhibited the germination of *B. integrifolia* and *Lomandra longifolia.* Therefore I suggest that both the indigenous acacia and exotic bitou bush have the potential to chemically inhibit the establishment of indigenous plants, with an additive effect. Eventual monoculture formation by bitou bush is likely to be facilitated by allelopathy against indigenous species and the residual soil inhibition of dominant *A. longifolia* var. *sophorae* establishment.

To determine whether bitou bush exuded novel compounds into the soil that were not present in the acacia dominated indigenous system, I compared the root and soil chemical profiles of these species. I focused on the hydrophobic extracts of the roots and soil as these were found to be most inhibitory in the laboratory based bioassays. Using solvent based extraction and gas chromatography – mass spectrometry (GC-MS) techniques, I 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*. Of particular interest were 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 and exhibited phytotoxic activity.

To confirm that bitou bush alters the soil chemistry of the sand dunes of the eastern Australian coast, we also designed a novel technique to assess the field soil chemical profile. The technique employed adsorbent resin filled bags intended to trap hydrophobic compounds in-situ which were then tested for bioactivity in the laboratory. I compared the hydrophobic chemical profile of soil below bitou bush and acacia to that of unvegetated soil. Similar GC profiles were found to those detected via the solvent extraction method; however, the resin bag technique showed that the alkane series was present in both the bitou bush and acacia soils. Using the resin bag technique, the chemical profile of the bitou bush invaded soil was characterised by a high concentration of sesquiterpenes and was distinct from the indigenous plant soil and bare sand, which were similar except for the presence of a higher concentration of phenolic compounds in the acacia soil and a higher concentration of hexadecanoic acid in the un-vegetated soil. Bioassays of these hydrophobic mixtures showed that the soil inhabited by plants, whether exotic or native, was inhibitory to the growth of an indigenous sedge, compared to the unvegetated soil.

Based on the series of experiments conducted, and described above, I suggest that the bitou bush invasion of the eastern Australian coast is likely to affect the recruitment limitation of indigenous species, rather than effects on fecundity and mature plant health. Bitou bush was found to induce a unique soil hydrochemical chemical profile, via two different techniques, which was characterised by high concentrations of several sesquiterpenes and low concentrations of a phenolic compound compared to the acacia profile. Although hydrophobic extracts both the bitou bush and acacia soils inhibited the growth of some indigenous species, the bitou bush inhibited more, including the dominant acacia, which is likely to result in the creation of vacant space and increased opportunities for bitou bush establishment and hence proliferation. Therefore, I suggest that allelopathy is

a key mechanism driving the recruitment limitation of indigenous flora and invasion of bitou bush on the eastern Australian coast.

Acknowledgements

Wow!!! What a huge experience!! I could write another thesis on the people I need to thank and the reasons why…maybe one day, but not now…so I will keep it brief.

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Chapter 1: General Introduction

1.1 The phenomenon of exotic plant invasion

1.1.1 Definitions

I refer to exotic invasive plants as those which have established, proliferated and spread, displacing indigenous resident species (Elton 1958; Mack *et al.* 2000; Colautti 2005). Other synonymous adjectives used in the literature include weeds, neophytes, aliens and non-indigenous plants. I use the term exotic or invader to describe species of foreign origin; and indigenous or non-invader, for endemic resident species of the location of interest. The term invasive is utilised to accentuate the spreading and detrimental nature of this subset of plants (Davis & Thompson 2000; Richardson *et al.* 2000). I also recognise the congruence of invasion ecology and broader ecological succession theories (Davis *et al.* 2001) as both drawing on species replacement patterns as guided by abiotic or biotic conditions. The sole distinction between succession theory and invasion ecology arises from the origin of the species: indigenous or exotic, which adds escape from enemies and the possible evolution of competitive ability as further possible mechanisms of replacement.

1.1.2 History and modes of exotic invasive plant introduction

The distributions and abundances of plant populations fluctuate spatially and temporally. Plant species distribution is governed by complex interactions between genetic capabilities and environmental conditions; the molecular interactions of which we are yet to fully understand (Bazzaz & Stinson 1999; Meyers & Bull 2002; Pigliucci 2005). Wallace's theory that plant distribution and abundance are guided by abiotic conditions, has

historically dominated plant population biology (Harper 1977; Silander & Antonovics 1982). Prior to the 1970's, pressures from other organisms, as suggested by Darwin's theory of the survival of the fittest (Darwin 1859), were overlooked in plant ecology theory (Harper 1967, 1977; Silander & Antonovics 1982; Shmida & Ellner 1984). Vertebrates, invertebrates, fungi and bacteria all have the capacity to affect plant species distribution and have been documented more recently, in the scientific and popular literature. For example, co-evolution between mycorrhizal fungi (Read 1991), frugivores or ants (Howe & Smallwood 1982) and plants offers mutual benefits to both parties. Therefore, the expanse and proliferation of plant establishment has a long history of cooperation with other organisms, including *Homo sapiens*.

From at least Neolithic times (ca. 6000BP) (Webb 1985), humans have carried seeds and fruits across vast distances and have therefore been significant dispersal agents for plants. As humans began to modify the land through fire and cultivation, environmental conditions were altered which inevitably favoured some species and prevented the establishment of others. Early historical records note that the rapid expansion of modern cultivation was paralleled by a rise in the establishment of vagrant species or weeds as noted by Aristotle (Aristotle 350BC). From 1500 AD as the technological advancements of the second millennium improved human global mobility, cultivation techniques and communication, the global landscape drastically altered (di Castri 1989). Vast areas of land were cleared to sow seeds transported from across the globe. Interesting and more nutritious species and cultivars were continually sought to feed an ever-expanding human population. Humans have not only acted as superior dispersal agents, but also manipulated the land both intentionally, and unintentionally, which has facilitated the establishment and

proliferation of exotic species (Manchester & Bullock 2000). Humans may now be considered the world's most powerful biotic selective force (Palumbi 2001).

Despite natural historians and botanists, such as Hooker and Darwin, commenting on the noticeable replacement of indigenous species by exotic species, particularly in New Zealand, during the late 18th to early 19th centuries, empirical investigations of exotic plant invasions only gained momentum in the mid 19th century (Inderjit *et al.* 2005). In the last few decades, increasing attention on invasion ecology has shown that some exotic and native species are expanding their original range and invading into areas where they were previously absent or in low abundance, displacing indigenous flora and fauna and altering entire ecosystems (Randall 1996). Key factors contributing to this biotic intercontinental transfer include anthropogenic introduction, escape from predators, the evolution of increased competitive ability, environmental change, and as hypothesized more recently, the inherent phenotypic and adaptive plasticity of successful species (e.g. Schweitzer & Larson 1999; Parker *et al.* 2003; Peperkorn *et al.* 2005). Species invasion is regarded as the second greatest threat to biodiversity behind land clearing (Vitousek 1992; IUCN 2000).

1.2 Impacts of plant invasion

1.2.1 Detection and difficulty of impact assessment

To determine whether a plant is exotic and invasive requires knowledge of the natural distribution of all plants and their time of arrival at various locations. Webb (1985) suggested that plants arriving at a location after 6000BP could be assumed to have foreign origins. However, the documentation of extant and past resident plant species, as well as the arrival time of new species are not comprehensive (Parker *et al.* 1999). Palynology can inform estimation of past vegetation composition, however the process is time consuming

and not widely engaged by botanists. Therefore, uncertainty over the origin of some plant species prevents comprehensive identification of exotic invasive species.

This uncertainty has ramifications in the assessment of invasion impacts. Most of the evidence for exotic plant impacts is anecdotal (Parker *et al.* 1999; Byers *et al.* 2002), although quantitative evidence is mounting (see 1.2.2 and 1.2.3). The identity of the exotic plant, including the habit, tissue chemistry, presence/absence of fruits and the similarity to native species, all influence the level and extent of impact. Which spatial scale is appropriate for assessment of invasion impacts? Some species can inflict a high impact over a short space, so that even a few individuals can have an effect, such as the toxic species *Robinia pseudo-acacia* (black locust) (Nasir *et al.* 2005). Other species may have a lower individual impact, particularly if they resemble a native plant in form and function, e.g. grasses. However if they have the capacity to displace other plants and form a monoculture, these latent threats can become equally as devastating. For example invasive graminaceous species *Bromus tectorum* alters indigenous species composition via positive feedbacks which reduce nitrogen availability (Evans *et al.* 2001). Plant invaders with long lag times between establishment and invasion also present difficulties in assessment as they may be adapting to the new environment or responding to a shift in environmental conditions prior to population explosion (Cousens & Mortimer 1995; Mack *et al.* 2000). Therefore, the prediction of potential impacts of an exotic species is complex. According to Byers *et al.* (2002) researchers have spent the last century trying to predict the impact of exotic species on resident species with only a 30% success rate. Despite many attempts to generalize the impacts of exotic invaders, species and site specificity reigns (Parker *et al.* 1999; Byers *et al.* 2002).

As Parker *et al*. (1999) note, generalisation of invasion impacts is not just an academic problem but also of management and policy concern. Which invasive species should be prioritized for control? Should we control invasive species on a species scale or a landscape scale? Which exotic plant invaders pose the greatest threat now and in the future and at what spatial scale? What framework can we use to gauge invasion impacts and therefore management priorities? Various models have been proposed, with most adopting a community based approach to impact assessment. For example Parker *et al.* (1999) suggest an impact score which incorporates the range, abundance and the effect per individual or biomass unit of the invader. The effect size is the controversial parameter where measurement is complicated by the potential for effects on all biotic and abiotic characteristics of an ecosystem. Difficulty in impact assessment is further exemplified if we consider the potential advantageous and detrimental effects of an invader on more than one species. *Lantana camara* has been shown to augment bird habitat (Crome *et al.* 1994; Date *et al.* 1996; Njorge *et al.* 1998) in an increasingly fragmented and exotic landscape. However *L. camara* also tends to form monocultures and displace indigenous vegetation (Stock & Wilde 2002). Indirect effects of invasion, where one species alters the effect that an exotic species has on a third species (Strauss 1991) has also not been widely researched (White *et al.* 2006).

Parker *et al.* (1999) advocate a Euclidean distance approach which allows comparison of multidimensional impacts in a single score between sites or for different species. They also acknowledge the use of bioindicators or biotic integrity models which have been critiqued by Simberloff (1997) as having too narrow a focus. To date, most empirical studies on invader impacts have been conducted on population and individual levels for not only plants, but also fish and invertebrates (Parker *et al.* 1999).

1.2.2 Effects on resident plants

Exotic invasive plants have the potential to affect all levels of plant organisation from genes, populations, species, and communities to ecosystems. Many plants depend on sexual cross pollination to persist and therefore, are genetically unique. Certain genotypes of a species may be more vulnerable to the environment created by exotic species invasion (Hoffmeister *et al.* 2005) resulting in reduced genetic variability of the population and the possibility of inbreeding depression or bottlenecks (Manchester & Bullock 2000). Alternatively, hybridisation between exotic and native species could occur, producing a superior invader (Williamson 1996) or sterile breeds which waste genetic resources (Trenham *et al.* 1998). Similarly, exotic plant invaders may drive the evolution of indigenous species traits such as the beak length of the soapberry bug (*Leptocorus tagalicus*) which has increased to accommodate feeding on the exotic balloon vine (*Cardiospermum grandiflorum*) (Carroll *et al.* 2005). Research into the genetic effects of plant invasion is still in its infancy however the premise for invasive plants to become evolutionary traps or forces of evolution (Schlaepfer *et al.* 2005; Mealor & Hild 2006) is clear.

The direct replacement of indigenous plants by exotics is a direct obvious impact of plant invasion, although there is a paucity of comprehensive quantitative research supporting the bulk of anecdotal evidence (Adair & Groves 1998; Parker *et al.* 1999). However empirical investigations demonstrating the displacement of indigenous species are accumulating (Weiss & Noble 1984a; Huenneke & Thomson 1994; Grant *et al.* 2003; Miller & Gorchov 2004). Studies on the effect of exotic invasive plants on resident plant population dynamics have primarily shown that exotic invaders disrupt the colonization or recruitment phase of resident plant life-histories (Merriam & Feil 2002; Grant *et al.* 2003;

Minchinton *et al.* 2006; Siemann & Rogers 2006) to the extent that Yurkonis and Meiners (2004) suggest colonisation limitation as a general mechanism driving species replacement by invasive plants. However this generalisation is equivocal as Howard and Goldberg (2001) found that the extinction rates of resident plants can also be increased as a consequence of invasion and others have shown that fruit or seed set can be reduced (Gould & Gorchov 2000; Miller & Gorchov 2004). Differences in the responses of spatially separated plant populations to an invader are also postulated based on the genetic differentiation of species at different locales, or localised adaptation (Joshi *et al.* 2001; Hobbs & Yates 2003).

On a larger scale, some successful invaders have been shown to alter entire plant communities by reducing species diversity (Maekawa & Nakagoshi 1997; Dunbar & Facelli 1999; Alvarez & Cushman 2002; Merriam & Feil 2002) and species richness (Costello *et al.* 2000; Meiners *et al.* 2001; Alvarez & Cushman 2002; Yurkonis & Meiners 2004). The importance of sampling at multiple sites is also highlighted by findings of site specificity of impact (Meiners *et al.* 2001; Wilkie *et al.* 2007).

Investigation of the indirect effects of exotic plant invasion is still rudimentary. Indirect effects can emerge due to the loss of the native species and its function in the system or conversely, due to the adverse effects of the habitat induced by the exotic plant. Recent studies show negative effects on native bird communities (French & Zubovic 1997), invertebrate (Lindsay & French 2004b; Wilkie *et al.* 2007), and microbial (Allsopp & Holmes 2001; Kourtev *et al.* 2002; Callaway *et al.* 2003a; Duda *et al.* 2003; Mummey & Rillig 2006; Reinhart & Callaway 2006) communities. These detrimental impacts of plant invasion result in reduced biodiversity which is directly related to ecosystem (Naeem *et al.* 1994; Chapin *et al.* 2000) and planetary sustainability (Whipple 1997). Ecosystem effects

are primarily driven by invasion induced changes to the abiotic parameters of the ecosystem which can ramify through the food web.

1.2.3 Effects on ecosystem function

The abiotic effects of exotic plant invasion are increasingly recognized as important impacts and mechanisms driving invasions. Since Vitousek and Walker's (1989) seminal study on the nitrification of Hawaiian soils invaded by *Myrica faya*, a flurry of studies into the effects of plant invasion on ecosystem properties have emerged in the literature. Effects of invasions on nutrient cycling have been are the most common, particularly in relation to nitrogen (Kourtev *et al.* 1999; Ehrenfeld *et al.* 2001; Evans *et al.* 2001; Siemann & Rogers 2003; Yelenik *et al.* 2004; Lindsay & French 2005; Knight *et al.* 2007). Recently, Ehrenfeld (2003) surveyed the literature in attempt to draw generalizations on the effect of plant invasions on nutrient cycling. Although she found a trend for plant invaders to increase the standing vegetation biomass, net primary production, nitrogen availability, decomposition rates and alter the nitrogen fixation rates compared to resident species, she also found evidence to the contrary, and differences between sites.

Exotic plants also may alter other chemical characteristics of the ecosystem including the soil organic chemistry (Langenheim 1994; Wardle *et al.* 1998; Hierro & Callaway 2003; Mitchell *et al.* 2006), availability of inorganic compounds (Marschener 1998), pH (Marschener 1998; Ehrenfeld *et al.* 2001) and water availability (Leege & Murphy 2001). Exotic plant invasion can also impact upon physical properties of the ecosystem such as temperature (Lindsay & French 2004a), light (Leege & Murphy 2001; Siemann & Rogers 2003; Reinhart *et al.* 2006) and fire characteristics (van Wilgen & Richardson 1985; D'Antonio & Vitousek 1992; Rossiter *et al.* 2003). Similarly, invasion

may also affect various structural characteristics of the ecosystem including the canopy or leaf litter architecture, soil porosity and soil aggregation; although direct studies of these potential effects could not be found in the literature.

By linking observed patterns of plant invasion impacts to the processes or mechanisms governing the change, we can gain a better understanding of the ecological and evolutionary implications of exotic plant invasion (Lavorel *et al.* 1999; Ackerley & Monson 2003).

1.3 Mechanisms of plant invasion

Our empirical understanding of the mechanisms driving invasion is limited (Prieur-Richard & Lavorel 2000; Levine *et al.* 2003; Olden & Poff 2003; White *et al.* 2006) and rarely incorporated into invasion impact studies (Levine *et al.* 2003). Ackerley and Monson (2003) recently highlighted the poor integration of physiology, plant function and evolutionary concepts into ecological theory. As a result, invasion mechanisms are broadly defined processes that have been accepted and cited as underlying causes with minimal morphological and particularly, physiological, biochemical or genetic clarification.

Here I propose a framework elucidating possible mechanisms of invasion which incorporates plant functional and evolutionary or plant attribute paradigms. I suggest a two tiered model founded on the "macro-mechanisms" or broad concepts existing in the literature, including exploitation, interference and space competition, which are necessarily buttressed by the "micro-mechanisms" of morphological, biochemical, physiological and genetic processes or plant attributes (Fig. 1.1). This conceptual framework of invasion mechanisms is congruent with community assembly rules derived from broad ecological theory. Community assembly rules are based on a spatial and temporal set of concepts

including dispersal, ecological (abiotic and biotic) filters, recruitment, interspecific interactions, abiotic effects (Begon *et al.* 1996; Crawley 1997). Within these determinants of community structure, plant attributes play a significant role (Noble & Slatyer 1981), particularly in the case of exotic plant invasion where one species has the potential to shape community composition. For example, testing of hypotheses regarding the micro-mechanisms involved in nutrient exploitation competition experiments will further our understanding beyond simple differences in leaf nutrient levels with and without a competitor, by asking: what plant attributes *allow* one plant to capture more nutrients than the other? Are genetic, physiological or morphological features enabling faster root growth? Does the plant have a greater capacity for faster root growth in the new home range? Is it a biochemical mechanism such as root exudation of organic acids which facilitates the bio-availability of nutrients? Higher enzyme levels facilitating faster incorporation of nutrients into molecules? A greater capacity for diffusion of nutrients across root cell membranes? Does the superior competitor inhibit the nutrient uptake of the other plant by secreting defense compounds into the soil? Or perhaps the superior competitor has all of these advantages and therefore the invasion utilizes macro-mechanisms of exploitative and interference competition.

It is highly likely that various macro-mechanisms are acting simultaneously, either in concert or opposition to one another (Vila & Weiner 2004; Inderjit *et al.* 2005) as depicted in Figure 1.1. However by ignoring the micro-mechanisms of plant-plant interactions, our understanding of invasion ecology will remain rudimentary. In a recent review of invasion mechanisms, Levine *et al.* (2003) found that when pitting exotics against natives (population level), exploitation competition was often cited as the mechanism driving the competitive outcome, however on community or ecosystem scales, ecosystem property

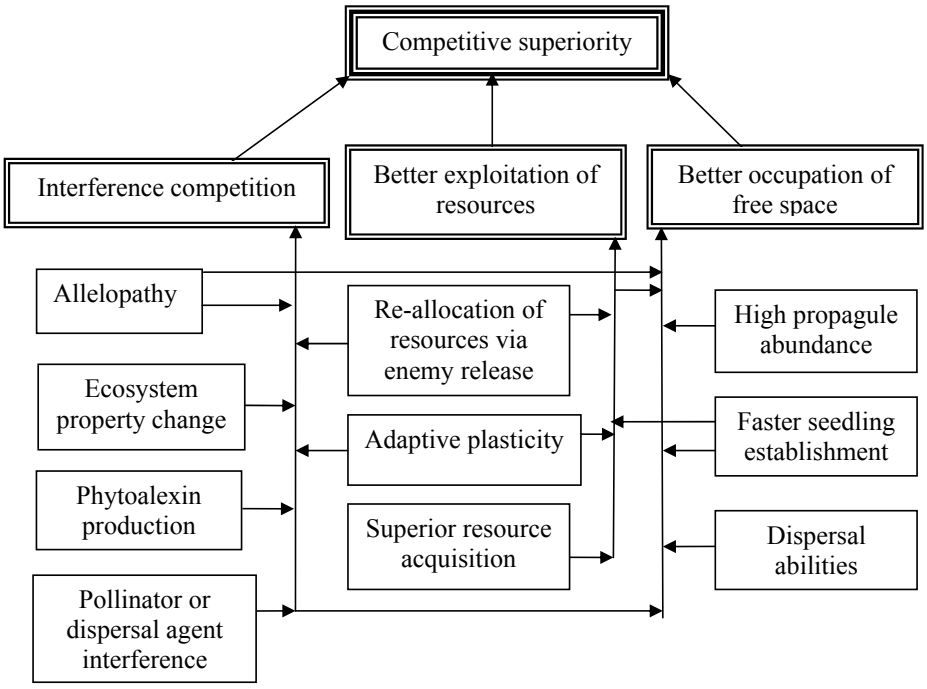


Figure 1.1: Conceptual framework for the macro (double lined boxes) and micro-mechanisms (single lined boxes) of invasion.

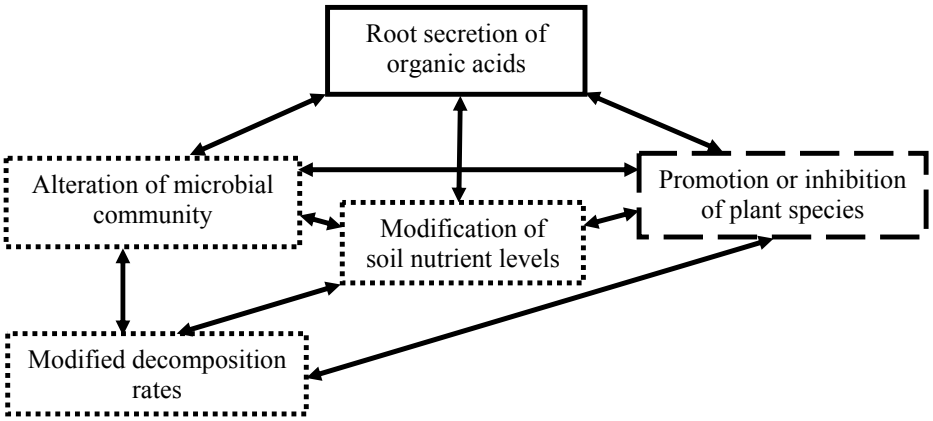


Figure 1.2: Flow chart highlighting the interactions between the micro-mechanisms (solid line) operating at a population level of detection (dashed line) and ecosystem property level (dotted line) as a result of organic acid secretion.

change was primarily implicated. Despite a discrepancy in the macro-mechanism identifier (exploitation and interference competition; Fig. 1.1), the common denominator is likely to be a micro-mechanism such as the secretion of organic acids (Fig. 1.2) which ramifies through the ecosystem suggesting exploitation competition at the population level and ecosystem property change at the ecosystem level .

The mechanisms of exotic plant invasion are often cited as exploitation competition (Levine *et al.* 2003) and enemy-release (Williamson 1996; Sax & Brown 2000; Mitchell *et al.* 2006). However exploitation competition is often insidiously intertwined with interference competition (Fuerst & Putnam 1983; Weidenhamer 1996), where both individuals suffer a net reduction in growth rate or one experiences an increase via interference on another species (Amarasekare 2002). Interference competition by an invader against new neighbours has been shown to be greater than interference against

species that have co-existed for a long time, which suggests that novel chemical weapons in the new range could be a key driver of some invasions (Hierro and Callaway 2003). The inherent adaptive plasticity (Schweitzer & Larson 1999; Parker *et al.* 2003) of invaders has also emerged recently as a macro-mechanism facilitating invasion and based on the framework presented here, would be a micro-mechanism which facilitates superior exploitation competition. Here I synergise our current knowledge of the macro-mechanisms of invasion and introduce extensions of these concepts to incorporate possible underlying morphological, biochemical, physiological and genetic micro-mechanisms.

1.3.1 Exploitation competition

Exploitation or resource competition is a direct negative interaction (against one or both individuals) based on the use of a common resource is often cited as the primary mechanism of invasion (Amarasekare 2002; Levine *et al.* 2003; Mitchell *et al.* 2006). The sedentary nature of plants necessitates efficient resource capture which is likely to have guided interspecific plant interaction theory to date. The outcome of interspecific plant interactions is often the desired result, with little investigation into the underlying mechanisms that drive the outcome (Levine *et al.* 2003). For example, superior light capture and swamping is often cited as a mechanism of plant invasion (Vitousek 1986; Williamson 1996; Levine *et al.* 2003; Coleman & Levine 2007). Although, the morphological micro-mechanisms underlying swamping, such as leaf area (Shainsky & Radosevich 2003) and root: shoot biomass (Glimskar & Ericsson 1999) are often assessed, the underlying physiological or genetic micro-mechanisms, such as those related to light energy absorption (e.g. chlorophyll content and density of light harvesting complexes)

which are essential for photosynthesis and plant growth, are rarely explored (but see Kraaij & Cramer 1999; Levine *et al.* 2003).

Water and nutrient acquisition is primarily reliant on plant uptake efficiencies, neighbour exclusion, loss prevention and use efficiency, all of which are genetically determined (Lambers & Colmer 2005). Uptake is dependent on the anatomical features of the root and root hairs that facilitate passive diffusion, active transport pumps and root searching capabilities, which must be highly adaptive based on the spatial and temporal heterogeneity of soil nutrients and water (Fitter 1997, 1999). Movement of nutrients through the soil is dependent on the soil buffering capacity, water content, structure and nutrient availability (Fitter 1997). If the exotic plant invader alters any of these properties, superior resource acquisition may detected, however the underlying causes may be overlooked if assessment of these soil properties is not assessed. Microbial associations are also often implicated in nutrient acquisition, and have the potential to affect competition outcomes between species. Therefore various micro-mechanisms could facilitate superior water or nutrient capture arising from genetic, chemical, physiological or morphological characteristics of the invader. Siemann and Rogers (2007) have recently published a similar criticism of interpretations of resource competition experiments, suggesting that studies claiming that an increase in tissue nutrient concentration is indicative of superior exploitation competition, may be overlooking the species specific requirements for that nutrient.

Furthermore, the process of exotic plant introduction and establishment into a new range can confer plants a competitive advantage based on the escape from natural enemies. The enemy release hypothesis (ERH) suggests that exotic plants have the potential for greater exploitation competition as they have escaped from co-evolved herbivores,

predators and pathogens and thus can re-allocate resources to further reproduction (Siemann & Rogers 2001), growth (Siemann & Rogers 2001; Genton *et al.* 2005) and therefore, survival (Darwin 1859; Elton 1958; Keane & Crawley 2002; Colautti *et al.* 2004). This latter point forms the basis of the evolution of increased competitive ability hypothesis (Blossey & Notzold 1995). When released from natural selection pressures, plants may do better or worse (Bossdorf *et al.* 2004), depending on species specific requirements and characteristics of the new host environment (Genton *et al.* 2005). Faster growth rates can also further benefit the exotic species if it introduces novel abiotic or biotic characteristics to the new host range. For example, if the exotic plant exuded allelochemicals in its home range, and demonstrated faster growth rates in the new home range, a relative increase in the amount of the exuded compound may be released in the new home range as a consequence of the faster growth rate.

However blind assumptions that invasive plants have geographically escaped natural enemies may be problematic, as studies have shown that some exotic plants experience increased herbivory (Carpenter & Cappucino 2005) and pathogen attack (Mitchell & Power 2003). Increased herbivory on exotic plants based on the absence of co-evolution and selection for increased enemy resistance, gave rise to the new associations hypothesis (Hokkanen & Pimentel 1989). Additionally, population bottlenecks as a result of low genetic variation in exotic populations that were initiated by a small number of individuals may also limit the adaptability of exotic species and confer reduced resistance to predators (Colautti *et al.* 2004). Therefore evidence for the ERH as a micro-mechanism of invasion is equivocal (Thebaud & Simberloff 2001; Keane & Crawley 2002; Genton *et al.* 2005; Mitchell *et al.* 2006), and further investigation of the explanatory physiological,

biochemical and primarily genetic processes are required to justify the ERH as a micro-mechanism facilitating exotic plant invasion.

The adaptive plasticity and potential rapid evolution of exotic plant invaders (Schweitzer & Larson 1999; Daehler 2003; Parker *et al.* 2003) have also recently emerged as possible micro-mechanisms facilitating competitive superiority and the possible displacement of resident plants. Organisms have been shown to respond to both changes in the mean and variability (Miner & Vonesh 2004) of abiotic resources (Stanton *et al.* 2000; Bray 2002), neighbour identity, or changes in herbivory (Bradshaw 1965; Bradshaw & Hardwick 1989; Reznick & Ghalambor 2001; Callaway *et al.* 2003b). Where developmental, adaptive or physiological plasticity exist, a population with a different mean in the plastic trait can evolve, giving rise to rapid evolution (Thompson 1991; Stockwell *et al.* 2003) as a result of restricted gene flow to individuals in the original habitat (Agrawal 2001). However, a mean change in a trait does not always constitute evolutionary adaptation - it may be that the developmental system is intrinsically labile and physiological shifts may not be transferred to the next generation (Meyers & Bull 2002). Additionally mutation in response to the stresses of the new environment may cause the rapid evolution of exotic species (Agrawal 2001; Meyers & Bull 2002). Genetic hybridisation between exotics and indigenous species has also been shown to benefit the invasiveness of exotic plants (Williamson 1996; Ellstrand & Schlerenbeck 2000).

The investigation of the underlying genetic, physiological and biochemical mechanisms facilitating morphological or physiological plasticity and the likelihood of consequent rapid evolution is a difficult task (Pigliucci 1996; Casal *et al.* 2004; Miner *et al.* 2005). Similar to the argument proposed for the ERH, assumptions that exotic plant exposure to a new range of selection pressures is likely to result in rapid evolution is

problematic. Identification of the precise environmental cue or cues inducing the change from a pool of many possible abiotic and biotic factors is challenging (Miner *et al.* 2005). Similarly, the exact biochemical and genetic pathways driving the observed physiological or morphological change are equally as complex (Casal *et al.* 2004). Further complication arises from the fact that many exotic invaders were originally introduced as ornamental plants or were selected for a purpose (e.g. a crop or pasture plant) and therefore vigorous genotypes were likely to be have been selected (Bossdorf *et al.* 2005). The introduction of vigorous genotypes with higher fecundity or larger leaves would result in populations expressing such trait means. By comparing plants from the home and introduced range we could therefore come to the erroneous conclusion that this species had evolved in response to enemy release or the new selection pressures. Therefore comprehensive assessment of the underlying genetic, physiological and morphological micro-mechanisms possibly responsible for the observed plasticity or evolution is required.

Despite the bias towards citations of exploitation competition as the mechanism of invasion in the literature, interspecific plant interaction in-situ, is likely to be a function of both exploitation and interference competition (Amarasekare 2002). Recent studies have demonstrated the co-occurrence of exploitation and interference competition and attempted to tease apart the relative importance of each as determinants of plant co-existence (Weidenhamer 1989; Nilsson 1994; Weidenhamer 1996). Interestingly Amarasekare's (2002) model of the parameters driving invasiveness, suggests that both exploitative and interference competition are likely to drive superior invaders, which is supported by empirical evidence from extensive studies with *Centaurea maculosa* (Ridenour & Callaway 2001). Examination of the possible micro-mechanisms featured in Figure 1.1 is likely to further assist in the distinction between these two processes.

1.3.2 Interference competition

Interference competition involves the direct or indirect effects between plants, primarily via changes to the edaphic and microclimatic conditions, atmospheric chemistry (e.g. Roshchina 1996), pollinators (e.g. Brown & Mitchell 2001) or seed dispersal agents (e.g. Meiners 2007). Plant invader alterations to the soil biotic and abiotic components are the most commonly published forms of interference competition which is often termed ecosystem process change (Vitousek & Walker 1989; Gordon 1998; Parker *et al.* 1999; Ehrenfeld 2003) or ecosystem engineering (Minchinton *et al.* 2006; Badano *et al.* 2007).

Exotic plant invasion can alter the soil microbial community (Sturz *et al.* 2001; Kourtev *et al.* 2003; Mummey & Rillig 2006; Reinhart & Callaway 2006) which in turn can induce negative feedbacks against resident plant communities (Barazani & Friedman 2001). However rarely are the underlying forces of the microbial community change explored. The associated micro-mechanisms that could potentially alter the microbial community include antimicrobial leachates (phytoalexin production) (Nilsson *et al.* 1993; Brimbecombe *et al.* 2001; Pieta & Patkowska 2001; Paterson *et al.* 2006), change in ground incident light, change to soil moisture or nutrients or the stimulation of different microbes.

The abiotic mechanisms facilitating plant invasion include the release of allelochemicals; and the alteration of microclimatic parameters (Meekins & McCarthy 2001; Lindsay & French 2004a), essential nutrients such as nitrogen (Vitousek & Walker 1989; Kourtev *et al.* 1999; Ehrenfeld *et al.* 2001; Evans *et al.* 2001; Lindsay & French 2005), soil physical properties, and dynamics of the leaf litter layer (Barritt & Facelli 2001; Minchinton *et al.* 2006). The underlying micro-mechanisms driving invasion via changes to the leaf litter layer could include the leaching of stimulatory or inhibitory chemicals, the

mechanical impedance of the leaf litter, the elemental composition of the leaf litter and the microhabitat changes associated with alteration of the leaf litter layer; many of which were investigated in a comprehensive study on the effects of *Casuarina pauper* litter on the growth of understorey species by Barritt and Facelli (2001).

Plant-derived compounds can drive vegetation composition via mechanisms of both resource competition and interference competition. Plant derived compounds include exudates actively secreted by leaves and roots, volatile compounds diffusing through leaves and roots, and the breakdown products of decaying and dead roots, leaves, flowers, fruits and seeds (Waller & Feng 1996); all of which may also be altered by, or alter the microbial community (Brimbecombe *et al.* 2001; Pieta & Patkowska 2001; Paterson *et al.* 2006) resulting in indirect chemical effects. The case whereby plant exudates or breakdown products directly affect the growth and development of other plants is known as allelopathy (Molisch 1937). Many studies invoking allelopathy as a mechanism of plant invasion have received criticism for the "grind and find" methodology which lack field based applicability. However such criticism has encouraged advocates of allelopathy to design eloquent experiments which clearly demonstrate the physiological, genetic and biochemical micro-mechanisms that underlie allelochemical exudation (Bertin *et al.* 2003; Inderjit & Duke 2003; Walker *et al.* 2003), movement through the soil (Inderjit 2001; Kobayashi 2004), uptake by plants (Glass & Bohm 1971; Lambers & Colmer 2005) and subsequent physiological (Einhellig 1986; Inderjit & Dakshini 1992; Einhellig 1995; Nimbal *et al.* 1996; Einhellig 2002; Inderjit & Duke 2003; Nishida *et al.* 2005; Lara-Nunez *et al.* 2006), genetic (Nishida *et al.* 2005), biochemical (Lara-Nunez *et al.* 2006) and morphological (Abdul-Rahman & Habib 1989; Inderjit & Dakshini 1992; Al-Humaid & Warrag 1998; Grant *et al.* 2003; Bonanomi *et al.* 2005; Nasir *et al.* 2005) effects. Perhaps the most

comprehensive study of allelopathy is demonstrated by the putative allelochemical, sorgoleone from *Sorghum bicolor*. Sorgoleone is the primary constituent of *S. bicolor* root exudates (Nimbal *et al.* 1996; Czarnota *et al.* 2001) which is produced in the root hairs and deposited between the plasmalemma and cell wall (Czarnota *et al.* 2001). Sorgoleone acts to inhibit plant growth by binding to the Q (8) binding site of the photosystem II complex (Czarnota *et al.* 2001), is a mitotic inhibitor (Hallak *et al.* 1999) and inhibits electron transport in photosynthesis and respiration (Einhellig *et al.* 1993; Nimbal *et al.* 1996). A number of plant species have been shown to be susceptible to *S. bicolor* root exudates (Nimbal *et al.* 1996; Czarnota *et al.* 2001; Erickson *et al.* 2001).

1.3.3 Occupation of free space

Better colonization and occupation of free space is suggested here as an individual mechanism of invasion which operates at the regional scale, in contrast to exploitation or interference competition which occur at the local or site scale (Tilman 1997). Here we also distinguish free space competition from exploitation or interference competition based on the underlying micro-mechanisms of propagule abundance, dispersal abilities and faster seedling growth (Fig. 1.1). A large abundance of propagules and faster seedling growth rates may be upshots of enemy release (Scott 1996), adaptive plasticity and superior resource acquisition (micro-mechanisms of exploitation competition), however, we see these attributes as exclusive advantages for free space occupation which would confer competitive advantage to an invader at early stages of establishment rather than later stages which are implicated in the macro-mechanism of exploitation competition. Although propagule pressure and dispersal are regarded as important influences on invasion success (Williamson 1996), there is a paucity of empirical evidence for or against (Lonsdale 1999).

Of the few studies on free space competition as a mechanism of invasion, high propagule abundance (Tilman 1997; Mason *et al.* 2007) and broad or rapid dispersal mechanisms including flooding (Florentine & Westbrooke 2005) and ocean currents (Batianoff 1997), appear to influence invasion success. Further research into the micro-mechanisms facilitating the superior occupation of free space by successful exotic plant invaders is therefore required to supplement our understanding of exotic plant invasion success.

1.4 Bitou bush invasion in Australia

Figure 1.3: Bitou bush in flower and fruit (left); invading coastal hindunes (centre); and invading coastal fordunes (right).

1.4.1 History of invasion

Bitou bush (*Chrysanthemoides monilifera* spp. *rotundata* (D.C.) Norl. (Fig. 1.3) is a perennial shrub in the Asteraceae family, of South African origin, which was first recorded in Australia near Newcastle in 1908 (Gray 1976) where it was thought to have been introduced through the dumping of ballast water (Cooney *et al.* 1982). During 1946 to 1968

the Soil Conservation Service of New South Wales extensively planted bitou bush to stabilize sand dunes (Mort & Hewitt 1953), especially following sand and rutile mining on the coast (Barr 1965) and on isolated inland dune systems (Cunningham *et al.* 1981). However bitou bush spread into relatively intact indigenous vegetation, and a recent survey found that 80% of the NSW coastline has been invaded with bitou bush (Thomas & Leys 2002).

1.4.2 Biodiversity impacts

Bitou bush has empirically been shown to alter ecosystem processes (Lindsay & French 2004a) and indigenous invertebrate (French & Eardley 1997; Lindsay & French 2004b; Wilkie *et al.* 2007), bird (French & Zubovic 1997) and vegetation communities (Weiss 1984; Weiss & Noble 1984b; Brewer & Whelan 2003; Mason *et al.* 2007). At least 63 plant species are thought to be threatened by the invasion of bitou bush (DEC 2006) and further vulnerable species have been identified (Mason 2007). As a result of the extensive impact and invasibility of bitou bush in Australia, it is regarded as weed of national significance (Agriculture and Resource Management Council of Australia & New Zealand *et al.* 2000) and a key threatening process under the New South Wales *Threatened species Conservation Act 1995* in 1999.

1.4.3 Mechanisms of invasion

There is some evidence to suggest that bitou bush invasion is facilitated by superior resource use and capture. Lindsay and French (2004a; 2005) have shown that bitou bush invaded areas were characterized by faster leaf litter decomposition rates and higher levels of soil nitrogen than found in native areas, suggesting the rapid cycling of nitrogen which is

likely to facilitate plant growth. Similarly, superior resource competition was suggested by Weiss and Noble (1984a) who found that bitou bush seedlings have a higher leaf area facilitating greater light capture, higher leaf chlorophyll content and greater root mass than seedlings of the dominant resident plant, *Acacia longifolia* which could facilitate faster uptake of water and carbon acquisition. Further investigations of the micro-mechanisms underlying these findings are, however, required to confirm exploitation competition as the reason behind these observations and exclude the possibility of interference competition. For example, higher soil nitrogen could be an indirect effect of microbial community changes or the release of nitrogenous exudates. Faster water uptake could be due to the exclusion of other plants roots by the release of allelopathic compounds, faster growth rates conferred by the allocation of resources in response to enemy release or a function of the age of the individuals (seedlings) studied.

Anecdotal and rudimentary evidence also suggests that bitou bush invasion is facilitated by allelopathy. Bitou bush litter has been shown to inhibit the germination and seedling growth of *A. longifolia* (Vranjic *et al.* 2000), and the germination of *Hardenbergia comptoniana* and *Lepidium sativum* (cress) (Hughes 1998). However these results could also have been due to the mechanical impedance or microclimatic changes induced by the litter layer itself, as noted by these authors. Seedling growth of *A. longifolia* was lower in bitou bush soil than in *A. longifolia* soil, although not significantly different (Vranjic *et al.* 2000) and aqueous leaf leachates have been shown to affect the germination and seedling growth of *Schoenia filifolia, Lepidium sativium* (cress) (Hughes 1998) and three woody heath species (*Eucalyptus viminalis*, *Hakea dactyloides* and *Casuarina littoralis*) (Copeland 1984). Aqueous root extracts were also found to affect the germination and seedling growth of the woody heath species, however these latter extract studies followed the "grind and

find" method which does not have field relevance. Copeland's (1984) study also had low sample sizes and germination success in controls. Although these studies suggest that allelopathy may be a mechanism facilitating bitou bush invasion, inconclusive results and inadequacies in experimental design and success hamper certainty. Further comprehensive investigation into the likelihood of allelopathy as a mechanism of plant invasion is therefore warranted.

1.5 Research aims

Displacement of resident plant populations by exotic plant invaders has been suggested to generally occur at the colonization or recruitment stage in the life cycle (Yurkonis & Meiners 2004), however there is some evidence that mature plant reproductive output and growth are also affected by invaders (Howard & Goldberg 2001). Identification of the vulnerable stages of resident plant life histories to the effects of an invader can direct management strategies to aid restoration goals. Mean differences between plant morphological and physiological traits in invaded habitats compared to non-invaded habitats is suggestive of invasion impact. However, differences between the variability within traits between invaded and non-invaded habitats indicates the presence of selection pressure the potential for acclimation of species traits (Callaway *et al.* 2005; Carroll *et al.* 2005; Hoffmeister *et al.* 2005). In order to investigate these ideas further, I asked the following questions:

1. Does bitou bush invasion alter the physico-chemical status of the invaded ecosystem?
2. Which life history stages of indigenous plants are most affected by the invasion of bitou bush?

3. Are the reproductive output, growth and health of mature plants affected by bitou bush invasion?
4. Are mature resident plants physiologically stressed by the invasion of bitou bush? And are some seasons more stressful for resident of invaded habitats compared to non-invaded habitats?

To further elucidate whether allelopathy is a mechanism of bitou bush invasion which contributes to the recruitment limitation of resident species, I explored the following questions:

5. Do extracts from bitou bush leaves, roots and soil affect the germination or seedling growth of indigenous species more than comparable extracts from the dominant indigenous plant of the system, *Acacia longifolia* var. *sophorae* (acacia)?
6. Which hydrophobic compounds are released by bitou bush and acacia roots into the soil? Do mixtures of these compounds affect the germination and seedling growth of indigenous plants?

1.6 Thesis outline

Chapters 2 to 6 contain independent manuscripts which have been submitted for publication in academic journals. Therefore, some repetition between chapters may exist. Chapter 2 describes the effects of bitou bush invasion on the life history stages of several indigenous plants, particularly, traits of the mature reproductive stage. Chapter 3 outlines the seasonal photosynthetic patterns of several indigenous plants and the physico-chemical characteristics of invaded and non-invaded habitats. Chapter 4 contains a comparison of the effects of bitou bush and acacia extracts on the germination and seedling growth of five indigenous species and an internationally adopted test species. A comparison of the

hydrophobic chemical composition of bitou bush and acacia roots and soil are presented in Chapter 5. I developed a new rapid technique for capturing hydrophobic compounds in the soil and tested mixtures from bitou bush soil and acacia soil and unvegetated soil on an indigenous test species which is described in Chapter 6. A summary and integration of my findings with current literature and future directions are provided in Chapter 7.

Journals each chapter have been, or will be, submitted to:

Chapter 1: Diversity and Distributions – accepted with revisions

Chapter 2: Functional Ecology - submitted

Chapter 3: Functional Ecology

Chapter 4: Journal of Chemical Ecology – submitted

Chapter 5: Soil Biology and Biochemistry

Chapter 2: Exotic woody invader limits the recruitment of three indigenous plant species.

2.1 Introduction

While interspecific interactions between plants occur whether plants are indigenous or exotic, an exotic plant may elicit novel changes that negatively affect indigenous plants (e.g. Vitousek *et al.* 1987; Bertness & Callaway 1994; Siemann & Rogers 2003) which may result in positive feedbacks that promote invasion success. Exotic plants can transform (sensu Richardson *et al.* 2000) habitats by altering fire regimes (D'Antonio & Vitousek 1992; Rossiter *et al.* 2003) and modifying the abiotic (Vitousek *et al.* 1987; Siemann & Rogers 2003; Lindsay & French 2004a) and biotic environment (Minchinton *et al.* 2006). Exotic plants may also influence neighbouring individuals by exuding novel phytotoxins (allelopathy) (Nilsson 1994; Ridenour & Callaway 2000; Amarasekare 2002; Hierro & Callaway 2003). These factors are likely to affect the survival of indigenous plant species which may have ecological and evolutionary implications for the invaded system (Hoffmeister *et al.* 2005).

Investigating the response of indigenous plant life history stages to exotic plant invasion can elucidate how an exotic species might limit the persistence of indigenous species (Howard & Goldberg 2001; Yurkonis *et al.* 2005). Analysis of the population size (or age) structure allows insight into life-history stages that are susceptible to invasion. If the mature stage of a species is affected by the invader, reproduction is likely to be reduced. Alternatively, recruitment limitation may occur through interference at the germination and seedling growth stages (Harper 1977; Siemann and Rogers 2006). Different life history stages of different species may be vulnerable to different neighbours (Howard and

Goldberg 2001) or there may be a similar effect on a particular life history stage across many species, for example seedling survival (Standish *et al.* 2001). Yurkonis *et al*. (2005) suggest establishment or recruitment limitation as a general mechanism of invasion impact, however further studies are required to substantiate this claim. Additionally, the replacement of even one indigenous species may have ecosystem level ramifications if the species played a significant role in the habitat e.g. a primary canopy species (Totland & Esaete 2002).

Despite much study into the population dynamics and autecology of exotic invaders (e.g. D'Antonio 1993; Paynter *et al.* 2003), the influence of exotic plant invasion on the demography of indigenous plants has received minimal investigation. Size-symmetric competition between similarly aged exotic and indigenous plants is commonly found in situations where an exotic plant establishes and competes with indigenous species following disturbances such as fire or land clearing. Size-asymmetric competition between plants occurs more frequently when an exotic plant invades into a heterogeneous vegetation community where indigenous species populations are represented by a range of individual sizes or ages. Therefore, the exotic invader is likely to encounter a range of life-history stages from seeds to mature reproductive individuals, and possibly exert differential effects on each stage, known as size dependent effects (Samson and Werk 1986). For example if the invader is a strong competitor for space and light smaller individuals may be more affected than larger individuals (Vitousek 1986; Gould & Gorchov 2000). To date, most demographic effects of exotic invasion have investigated the effects on germination and seedling growth of indigenous species (Weiss & Noble 1984a; Walker & Vitousek 1991; Vranjic *et al.* 2000; Gorchov and Triesel 2003; Grant *et al.* 2003). Furthermore, there is a bias towards invader effects on annuals and herbs (e.g. Gould & Gorchov 2000; Bakker &

Wilson 2001; Grant *et al.* 2003) rather than woody shrubs and trees (but see Weiss & Noble 1984a; Fogarty & Facelli 1999; Standish *et al.* 2001; Gorchov & Trisel 2003). Few studies have investigated the impact of invasion on mature plant reproductive (but see Gould and Gorchov 2000; Standish *et al.* 2001;Miller and Gorchov 2004) and survival capacity (but see Gould and Gorchov 2000; Miller and Gorchov 2004). Two studies have shown that reproductive output and growth of transplanted seedlings of annuals (Gould and Gorchov 2000) and transplanted perennial herbs (Miller and Gorchov 2004) were lower in *Lonicera maackii* invaded plots compared to transplants in plots were the invader was removed. The effect was seen within a year of transplanting the annuals, and two years after and subsequent years after transplanting for two perennial herbs. Conversely, the fecundity one of the perennial species in Miller and Gorchov's study (2004) was not affected by the presence of the invader compared to removal plots, and Standish *et al.* (2001) found that the seed rain of forest canopy species was not affected by the invasion of a ground cover, *Tradescantia fluminensis.* From these studies, we can assume that if there is going to be an effect of exotic plant invasion on the reproductive output of resident species, we would expect it to occur soon after the invasion.

For mature plants, trade-offs between reproduction and growth are common responses to different environmental conditions (Harper 1977; Kawano & Masuda 1980; Fynn *et al.* 2005). These responses require shifts in resource allocation and adaptive plasticity at the individual level which is likely to be an important determinant of indigenous species persistence in invaded habitats (Bradshaw 1965; Sugiyama & Bazzaz 1997). While plasticity in response has been investigated for environmental disturbances such as pollution (Ling 2003; Zvereva & Kozlsov 2005) and drought (Sanchez-Gomez *et al*. 2006), there has been no direct investigation into the adaptive plasticity of indigenous

plants in response to plant invasion. When exposed to unfavourable environmental conditions, polycarpic perennial plants are expected to direct resources towards persistence and growth rather than reproduction (Harper 1977; Crawley 1997). Bradshaw (1965) also acknowledged that the number of flowers produced may be a plastic feature as they require long periods of meristematic activity which are susceptible to environmental fluctuation. We expect that there might be differences in both the mean population responses and variability between individual responses of plants occurring in invaded habitats. If the invasion is recent we might expect an increase in the variability of specific traits between individuals, as different individuals are likely to respond differently to the same environmental change (Gutschick & BassiriRad 2003), which would indicate that adaptive plasticity may be occurring in some plants. If the invasion is long standing and natural selection, or loss of intolerant individuals has occurred, we might expect a difference in the trait means between invaded and non-invaded habitats.

The aim of this study was to examine how the invasion of exotic bitou bush (*Chrysanthemoides monilifera* spp. *rotundata* (DC.) T. Norl.) affected the demography of perennial indigenous *Correa alba* var. *alba* (Andr.) , *Monotoca elliptica* ((Sm. (R. Br.)), *Lomandra longifolia* (Labill.) populations on the eastern Australian coast and ascertain which life history stages were most susceptible. We compared the population size structure and density of these three species in bitou bush invaded and non-invaded habitats. We assessed whether the invasion had an effect on mature plants by comparing flower production, the number of vegetative buds, the ratio of reproductive: vegetative buds and the physiological stress levels of the indigenous species in invaded and non-invaded habitats. We also determined if the variability in the expression of these traits differed

between the invaded and non-invaded habitats as an estimate of adaptive plasticity of these species.

2.2 Methods

2.2.1 Study location and study species

The study was undertaken on coastal dunes between Kurnell (34˚0'S 151˚21'N) and Moruya (35˚91'S 150˚15'N) in New South Wales, Australia. Across this latitudinal range, mean annual maximum and minimum temperatures ranges between 20.4˚-22.1˚C and 11.3˚-13.3˚C respectively and mean annual rainfall ranges from 961-1094mm (Australian Bureau of Meteorology 2006). Rainfall is highest from January to April. The study area is characterized by Holocene sand exposed beaches with parallel sand dune systems. Typical species found on the fore dune include *Spinifex sericea, Acacia longifolia* var. *sophorae, Lomandra longifolia, Leucopogon parviflorus, Correa alba* var. *alba* and *Carprobrotus glaucescens.* The hind dune is characterized by *Leptospermum parviflorus, Acacia longifolia* var. *longifolia, Banksia integrifolia, Lomandra longifolia, Monotoca elliptica* and *Eucalyptus botryoides.*

Five sites were selected to represent each of two habitat types: bitou bush invaded or non-invaded. Site selection required target species presence and minimal anthropogenic disturbance. Bitou bush invaded sites contained at least 50% cover of reproductively mature bitou bush at the time of study. All sites had been invaded for at least approximately 40 years despite efforts to control the invasions via Roundup® (Monsanto) application and manual removal (K. Thomson, D. Pomery and N. Dexter pers. comm) sporadically from 1994 to 1999.

To facilitate generalization of our findings, taxonomically and morphologically distinct indigenous species were chosen: *Correa alba* var. *alba* (Rutaceae), an endemic shrub of New South Wales sand dunes (Fig. 2.1.a); *Monotoca elliptica* (Epacridaceae), a medium sized tree found in the central eastern Australian hind dunes (Fig. 2.1.b); and a rush, *Lomandra longifolia* (Lomandraceae), which has a wide distribution from the eastern coastal sand dunes to inland eastern Australia (Fig. 2.1.c). All three species reproduce by sexual reproduction rather than vegetative propagation (*C. alba* var. *alba*: Auld 2001, Benson and McDougall 2001; *M. elliptica*: Benson and McDougall 1995; *L. longifolia*: Benson and McDougall 2005).

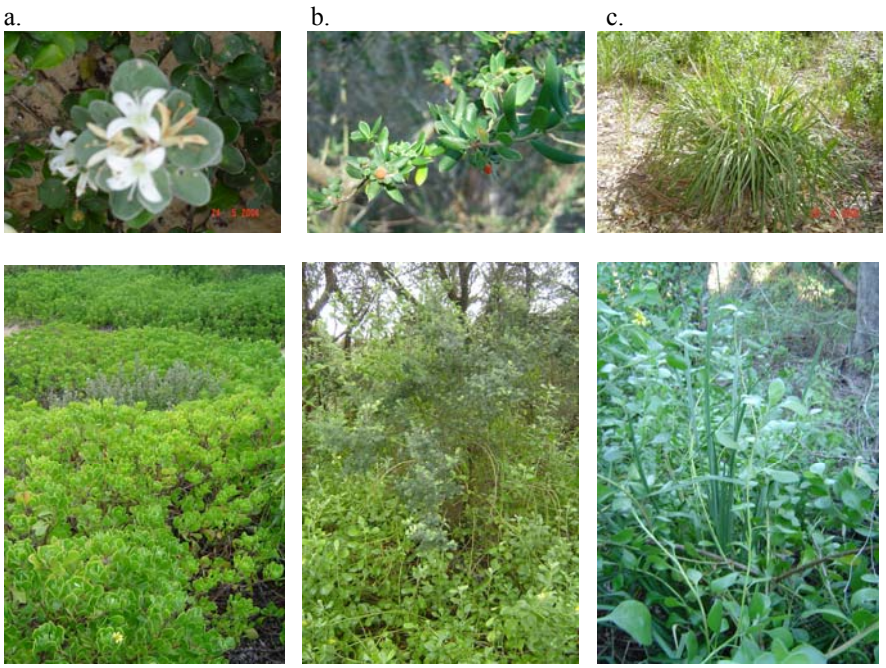


Figure 2.1: a. *C. alba* flower (top) and surrounded by bitou bush (bottom). b. *M. elliptica* in fruit (top) and surrounded by bitou bush (bottom). c. *L. longifolia* plant (top) and surrounded by bitou bush (bottom).

2.2.2 Population structure

The size of all individuals of each study species was measured in three random 100m^2 quadrats at each site in each habitat during January 2006. Size of *C. alba* and *M. elliptica* was assessed by measuring the diameter of the stem at 5cm and 10cm respectively above the root crown. The size of *L. longifolia* was determined by measuring the basal circumference of each plant. The number of individuals of each species in each quadrat was used to assess the density of each species in the bitou bush invaded compared to non-invaded habitat.

2.2.3 Morphological and physiological responses

To assess the morphological and physiological responses of each study species, five mature (flowering stage) individuals at least 5m apart were selected at each site, resulting in 25 plants per habitat type. From November 2004 (*M. elliptica* and *L. longifolia*) to May/June 2005 (*C. alba*), the total number of flowers and vegetative buds was counted for each plant. Flower production and vegetative buds for each species were distinguished visually based on the difference in colour and shape. When counts exceeded 100 buds, the number of buds was estimated as the average of three sub-samples multiplied by the reciprocal fraction of the plant volume to approximate for the whole plant.

The ratio of reproductive (number of flowers) to vegetative buds was calculated as an indicator of the trade-off between reproduction and growth. As size dependent (allometric) variation in the number of buds has been estimated (Samson & Werk 1986), we included the size of individuals as covariates in the analysis of flower and vegetative bud production and the trade-off between them.

Physiological plant stress was assessed by measuring the optimum photosynthetic yield, Fv/Fm, using chlorophyll fluorescence. The parameter Fv/Fm is linearly related to the efficiency of oxygen evolution by plants and a decline in Fv/Fm from the maximum of 0.83 is an indicator of physiological stress (Bjorkman & Demmig-Adams 1987). Five leaves were randomly collected from each plant and dark adapted in the field for at least 30 minutes. Fv/Fm for each leaf was then measured using a mini PAM (Pulse Amplitude Modifier) chlorophyll fluorometer (Heinz Walz, Effeltrich Germany).

2.2.4 Statistical analysis

The density and mean size of each study species in each habitat was compared using ANOVA. To assess the size distribution of the study species populations associated with both habitats we employed the Kolmogorov-Smirnov test (SPSS Version 12.01). The Kolmogorov-Smirnov test determined whether there was a difference between cumulative frequency distributions. Differences in mean flower production, vegetative bud abundance, reproductive: vegetative buds and the physiological stress of indigenous plants between habitats were compared using a mixed General Linear Model. Traits were ln (x +1) transformed to meet the assumptions of normality and homogeneity of variance and the size of each plant were included as a covariate in the model. Habitat type (bitou bush invaded or non-invaded) was considered a fixed factor and site was a random factor, nested within habitat. The F-test (Zar 1999) was used to determine whether there was a significant difference in the trait variation between habitats based on the amount of variation among sites (Variation among sites between habitats = Mean Square of the invaded sites/ Mean Square of the non-invaded sites) and/or whether there was more variation among plants

within sites (Variation within sites between habitats = Error Mean square of invaded habitat/ Error Mean square of non-invaded habitat).

2.3 Results

2.3.1 Population structure

There was a significantly greater density of *C. alba* ($F_{1,28}$=6.67, p=0.015), *M. elliptica* ($F_{1,28}$=15.53, p<0.001) and *L. longifolia* ($F_{1,28}$=14.29, p=0.001) in the non-invaded habitat compared to the bitou bush invaded habitat (Fig. 2.2).

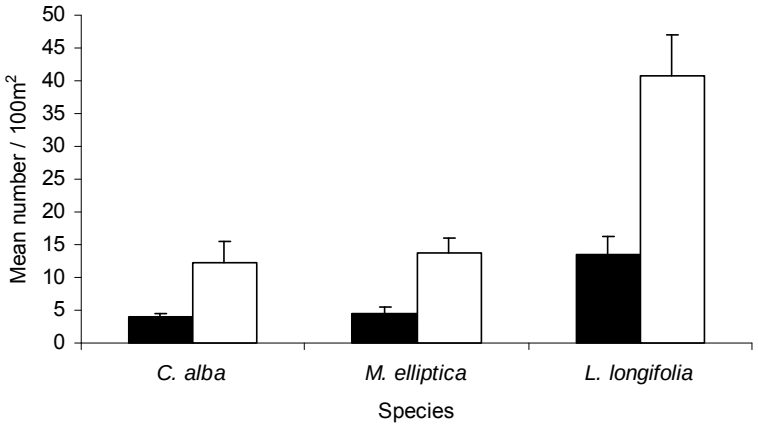


Figure 2.2: Mean density (+ SE) of each study species in bitou bush invaded (black bars) and non-invaded (open bars) habitats.

The *C. alba, M. elliptica* and *L. longifolia* populations in the bitou bush habitat were also significantly different in size structure compared to those found in the non-invaded habitat (Z=2.51, p < 0.001; Z=1.79, p=0.003; Z=3.32, p < 0.001). In the non-invaded habitat, all three indigenous species had a high number of small individuals, typical of

strongly rejuvenating populations (Agren & Zackrisson 1990), however in the bitou bush invaded habitat, all exhibited lower abundances of smaller individuals (Fig. 2.3).

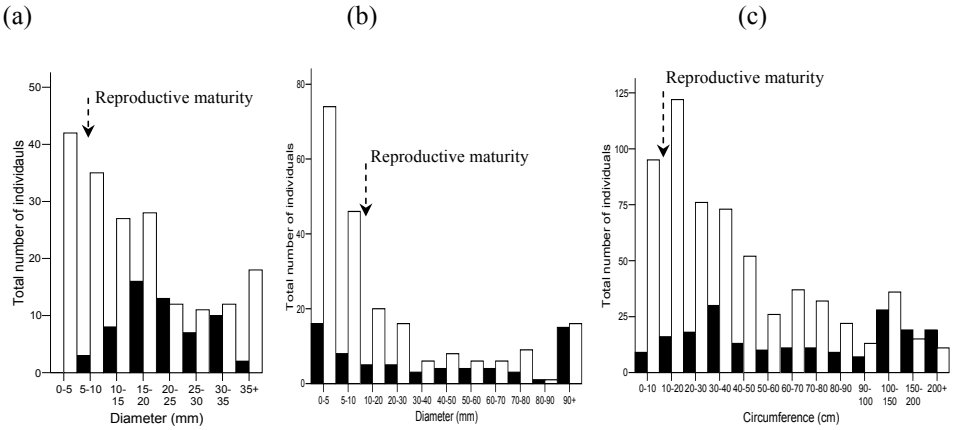


Figure 2.3: Frequency histograms showing the number of (a) *C. alba* (b) *M. elliptica* and (c) *L. longifolia* individuals within 1500m^2 of the bitou bush invaded (black bars) and non-invaded (open bars) habitats.

In the total area sampled in the non-invaded habitat (1500m^2) we found 42, 74 and 95 *C. alba*, *M. elliptica* and *L. longifolia* juveniles respectively, whereas in the invaded habitat we found a total of 0, 16 and 9 *C. alba*, *M. elliptica* and *L. longifolia* juveniles respectively which equates to a 100%, 78% and 91% difference in the number of juveniles between habitats. In the next three size classes there was a reduction in the difference between the number of individuals of each species between the two contrasting habitats: in the invaded habitat the number of *C. alba* plants went from 91% to 43%, *M .elliptica* from 83% to 69% and *L. longifolia* from 87% to 56% of the number found in the non-invaded habitat. We also found approximately double the number of mature individuals of all three species in

the non-invaded habitat compared to the bitou bush invaded habitat (Fig. 2.3). Furthermore, because the average sizes of mature plants were significantly greater in the invaded habitat compared to the non-invaded habitat for *L. longifolia* ($F_{1,704}$ = 58.83, p < 0.001) and *M. elliptica* ($F_{1,130}$ = 5.56, p = 0.02) this suggests that there were fewer smaller reproducing individuals in the invaded habitat. However there was no difference in the size of mature plants between habitats for *C. alba* ($F_{1,200}$ = 1.453, p = 0.229 (Fig. 2.4) suggesting that there are fewer mature *C. alba* individuals of all sizes in the invaded habitat.

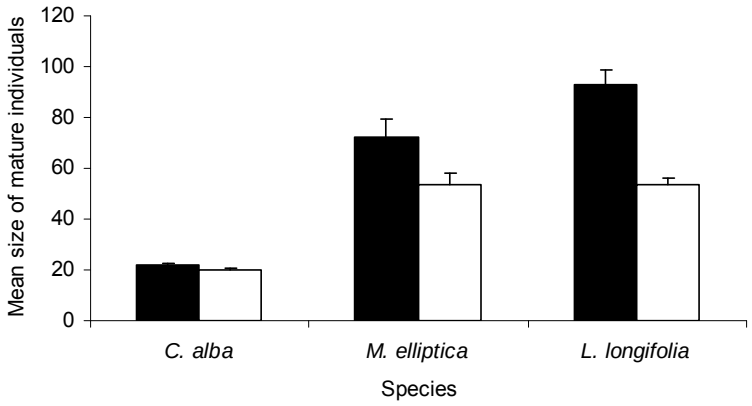


Figure 2.4: Mean (+ SE) size of the mature (reproductive) individuals of each species in the bitou bush invaded habitat (black bars) and in the non-invaded habitat (open bars). The size of *C. alba* and *M. elliptica* was measured as the diameter (mm) and the size of *L. longifolia* was measured as circumference (cm).

2.3.2 Morphological and physiological responses of mature indigenous species

The sizes of mature individuals sampled in the invaded and non-invaded habitats were similar for *C. alba* ($F_{(1,8)}$=7.27, p=0.42), *M. elliptica* ($F_{(1,8)}$=0.18, p=0.68) and *L.*

longifolia ($F_{(1,8)}$=0.13, p=0.29). The mean Fv/Fm, flower production, vegetative bud abundance and reproductive: vegetative buds of mature *C. alba, M. elliptica* and *L. longifolia* did not significantly differ between the non-invaded and bitou bush invaded habitats (Table 2.1).

Table 2.1: F ratios and p values of flower abundance, vegetative buds, the ratio of reproductive: vegetative buds and physiological stress traits (Fv/Fm) of each species between non-invaded and bitou bush invaded habitats.

	Flower abundance		Vegetative buds		Reproductive: vegetative buds		Fv/Fm	
Species	F (1,8)	p value	F (1,8)	p value	F (1,8)	p value	F (1,8)	p value
C.alba	0	0.983	0.76	0.390	0.02	0.880	0.32	0.576
M. elliptica	1.49	0.231	0.60	0.443	0.17	0.683	0.86	0.360
L. longifolia	0	0.963	0.19	0.666	0.14	0.898	0.04	0.551

Comparison of the variation of all measured parameters among sites and among individuals within sites between the bitou bush invaded and non-invaded habitats, revealed that there was a similar amount of variation between habitats for all traits across all species except the flower production and reproductive: vegetative buds of *C. alba* (Table 2.2). There was greater variation in the number of *C. alba* flowers between sites in the invaded habitat compared to the non-invaded habitat (Table 2.2; Fig. 2.5). There was also significantly more variation in the ratio of reproductive buds: vegetative buds within sites in the invaded habitat compared to within sites in the non-invaded habitat for this species (Table 2.2; Fig. 2.6). There was no consistent correlation with level of bitou bush invasion or physico-chemical parameters to suggest the likely cause of this variability (physico-chemical analysis presented in Chapter 3).

Table 2.2: Results of the F tests comparing the variability in traits among sites, and within sites, between the bitou bush invaded and the non-invaded habitats for *C. alba, M. elliptica* and L. *longifolia.*. * P < 0.05. Five sites in each habitat and five individuals in each site were assessed.

Species	trait	Variability among sites between habitats $F_{(4, 4)}$	Variability within sites between habitats $F_{(20, 20)}$
C. alba	Flower production	8.81 *	1.38
	Vegetative buds	1.50	1.64
	Reproductive: vegetative buds	5.42	2.80 *
	Fv/Fm	1.49	1.05
M. elliptica	Flower production	5.42	1.54
	Vegetative buds	1.45	1.80
	Reproductive: vegetative buds	5.07	0.82
	Fv/Fm	2.50	0.00
L. longifolia	Flower production	0.27	1.37
	Vegetative buds	0.63	0.64
	Reproductive: vegetative buds	0.13	2.03
	Fv/Fm	1.46	1.69

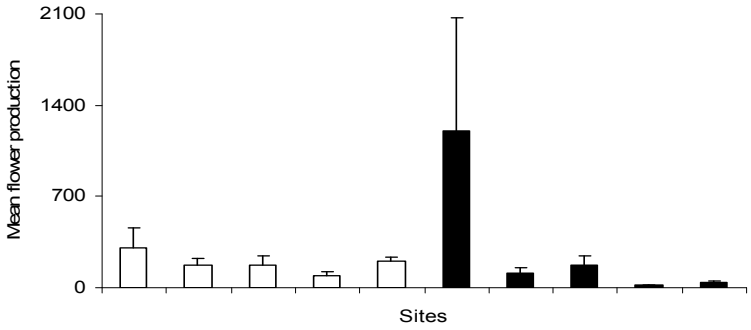


Figure 2.5: Mean (+SE) *C. alba* flower abundance at each site in the invaded (black bars) and non-invaded (open bars) habitats.

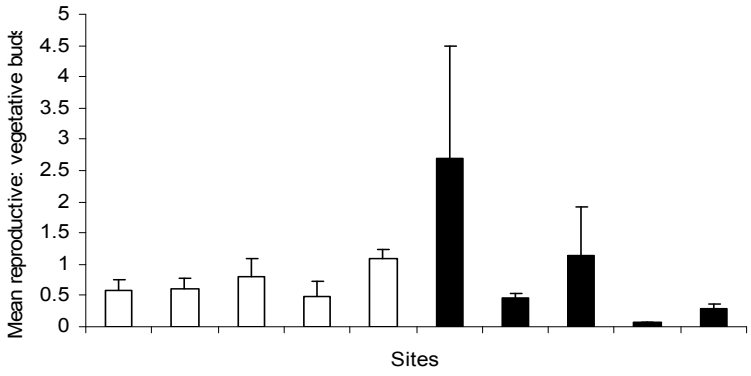


Figure 2.6: Mean (+SE) ratio of *C. alba* reproductive: vegetative buds at each site in the invaded (black bars) and non-invaded (open bars) habitats.

2.4 Discussion

Our findings suggest that bitou bush exerts dominance and ultimately forms a monoculture primarily by affecting the recruitment of indigenous species, rather than influencing the stress and reproductive capacity of mature plants. Two lines of evidence suggest this: the low abundance of juvenile individuals in the bitou bush invaded habitat and the similarity in flower and vegetative bud abundance and physiological stress of mature individuals in invaded and non-invaded sites.

C. alba, M. elliptica and *L. longifolia* populations present in invaded habitats lacked the characteristic high proportion of juvenile individuals found in non-invaded populations. The lower number of intermediate sized plants also suggests that the lack of juveniles in the invaded habitat is not due to faster growth of seedlings in the invaded habitat compared to the non-invaded habitat. This result therefore could suggest that there is a lack of seed input into invaded communities or it could represent a failure of seeds to germinate or establish.

We propose that the latter explanation is more likely as the abundance of flowers produced by the adult plants of the present study were similar across both invaded and non-invaded habitats and the indigenous species seed bank has also been shown to be relatively unaffected by bitou bush invasion (Mason *et al.* 2007), indicating that seed input is likely to be similar. However, if the invasion is not managed, the lack of juvenile plants and mortality of mature individuals is likely to result in reduced seed input into the sites in the future.

The snapshot of the population size structures gives insight into past recruitment. The increasing discrepancy between the number of individuals in the smallest four size classes between the invaded and non-invaded habitats indicates that in the invaded system there appeared to be less recruitment in the recent past compared to the level of recruitment found in the non-invaded habitat. This size distribution in invaded habitats suggests that there has been a lack of seedling germination in the recent past or an abnormal level of seedling mortality in the present invaded system. In the non-invaded population, for all three indigenous species, there was an exponential decline in the number of individuals of increasing size, suggesting that as seedlings matured, there was a pattern of mortality as competition, herbivory and environmental conditions limited individual survival. Previous work (Mason *et al.* 2007) found that the indigenous soil seed bank in hind dunes is largely intact although they found a significant reduction in tree species richness in the invaded sites compared to the non-invaded sites. This suggests that seedling and juvenile periods are likely to be the life history stages that are most affected by the presence of bitou bush. Furthermore, as the three indigenous species investigated were taxonomically and morphologically distinct, the impact of bitou bush may be ubiquitous and therefore induce a blanket effect on the regenerative capacity of the invaded community leaving space which

is likely to facilitate bitou bush monoculture formation. Recruitment limitation has previously been suggested as an important determinant of community structure (Tilman 1997) and specifically as a mechanism facilitating invasion success (Standish *et al.* 2001; Yurkonis *et al.* 2005; Minchinton *et al.* 2006; Siemann and Rogers 2006).

Yurkonis *et al.* 2005 have proposed establishment or recruitment limitation as a general mechanism for invasion impact. Several other studies support this hypothesis through two approaches: by showing that mature plant fecundity is not affected (Standish *et al.* 2001; Thomson 2005) and that recruitment is affected (Standish *et al.* 2001; Gorchov and Triesel 2003; Thomson 2005) by exotic plant invasion. Our studies also support this hypothesis as the invasion of bitou bush did not significantly affect the abundance of flowers produced by three morphologically distinct species (a small tree, shrub and rush) and the population size structure analysis suggested recruitment limitation at the germination and or seedling stage. Conversely, the growth and reproductive capacity of three native annual herbs (Gould and Gorchov 2000) and three perennial forest herbs (Miller and Gorchov 2004) were reduced by the invasive shrub *Lonicera maackii*. Hence it appears that although there is a trend for the recruitment stage to be more vulnerable to plant invasion, the habit of the invader and resident species may prevent broad generalizations on invader impacts. Additionally, other forms of disturbance may also be present in different systems which may confound or complicate interpretation of apparent effects.

In coastal dune environments, species tend to exhibit high stress tolerance as they have adapted to considerable environmental stresses such as salinity and low moisture and nutrient soil (Ernst 1985). Hence unless plants are exposed to an extreme environmental stress causing mortality (e.g. fire), mature individuals are likely to acclimate and modify

their survival strategy or resource allocation, or simply tolerate the stress. Different species and even different genotypes of the same species can respond differently to different stressors depending on their reaction norm (Sultan & Bazzaz 1993; Larcher 2003; Gutschick & BassiriRad 2003). Therefore, the nature and intensity of the response of individual plants to a stressor may vary depending on the size of the individual, its genotype and the influence of other environmental stresses. Hence we expected a range of individual responses to the bitou bush invasion, including tolerance.

The increase in variability of *C. alba* flower production among the invaded sites compared to the non-invaded sites suggests that at different invaded sites this plant responded differently to the invasion. At one invaded site in particular, *C. alba* produced a far greater number of flowers on average than at any other site, whereas a very low mean flower production was found at two invaded sites. This differential effect may be due to the clustering of different genotypes, different duration of invasion, or other forms of disturbance, such as different bitou bush control schemes at different sites that resulted in the observed site specificity of flower production for this species. At the individual level, we found that there was significantly higher variability in the ratio of reproductive: vegetative buds within invaded sites compared to within non-invaded sites. This further suggests that the invasion has different effects on different genotypes so that some, but not all individuals may be acclimating to the presence of the exotic by shifting their resource allocation. As there was more variability in the flower production of *C. alba* in the invaded habitat and no change in the number of vegetative buds, we conclude that the allocation to reproduction is likely to be the parameter which is expressing adaptive plasticity in this case. Hence it appears from our findings that mature *C. alba* genotypes respond differently to the bitou bush invasion by either increasing or decreasing resource allocation to

reproductive structures. The differential response of individuals of this species to the invasion resulted in no overall mean difference in flower production. This finding highlights the importance of assessing the variability in plant responses to environmental change as a tool for early detection of future impact. In the long-term, the loss of certain genotypes, in this case those that produced less flowers in invaded sites, may result in reduced genetic variability in the population. Maintenance of genetic variability is essential for the survival of species.

The invasion of bitou bush into the hind-dune woodland did not have any significant effect on the traits of the adult resident species studied, *M. elliptica* and *L. longifolia*, as the invaded habitat is likely to be similar to the non-invaded habitat which has a substantial woody species presence with a tall well developed canopy. The population size structure analysis also revealed that numbers of the largest mature individuals of both species were similar in the invaded and non-invaded habitats suggesting that the adults were not affected by the invasion that occurred decades ago. This finding is also supported by the significantly greater size of mature *C. alba* and *M. elliptica* individuals in the invaded habitat and the two-fold abundance of mature individuals in the non-invaded habitat which suggests that there has been a lack of recruitment for some time in the invaded habitat as there are fewer small individuals with reproductive capabilities. Comparatively, although there was approximately half the number of reproductively mature *C. alba* individuals in the invaded habitat compared to the non-invaded habitat there was no difference in the mean size of the population. This suggests that both the juveniles and adult individuals may drop out of bitou bush invaded systems or that the invasion predated the establishment of these larger individuals, and limited the recruitment of that older cohort. The former explanation is plausible based on our finding that individuals at some invaded

sites showed a marked decrease in flower production, suggesting suboptimal environmental conditions. With sustained invasion, these individuals may drop out of the system so that the tolerant or acclimated individuals remain. Unfortunately we do not know the precise date of the invasion or the size-age relationships of these species. Long term studies are hence required to elucidate the recruitment limitation thresholds for resident species this invaded system.

In both the fore-dune and hind-dune regions of the sand dune systems, the invasion of bitou bush alters the ground level microclimate (Lindsay & French 2004a). The increased moisture, decreased light and moderated temperature effect at the ground level is likely to affect the germination and seedling development of indigenous species which typically experience low moisture, high light and extremes of temperature. This invasion induced environmental change,coupled with our finding that adult plants appear to not be significantly affected by the invasion, suggests that the recruitment and establishment stages of indigenous plant life histories are predicted to be the stages that limit the success of indigenous plant populations in bitou bush invaded areas of the eastern Australian coast.

Therefore, the invasion of bitou bush on the eastern Australian coast is likely to inhibit the recruitment of juveniles in the short term, which will affect the persistence of indigenous communities in the long term. We found evidence to suggest that some indigenous species may have the inherent capacity to acclimate and tolerate to this new neighbour as a result of the adaptive plasticity exhibited by stress tolerant plants. However the long term survival of indigenous plants in bitou bush invaded habitats on the eastern Australian coast is likely to be hampered by the rapid pace of the bitou bush invasion and the high degree of plasticity typical of exotic plant invaders which may further facilitate invasive success (Mack *et al.* 2000; Hoffmeister *et al.* 2005). Understanding the

interactions between exotic plants and the life history stages of susceptible indigenous plants allows us to identify vulnerabilities in the indigenous community which could assist in their restoration.

Chapter 3: Seasonal photosynthetic patterns of mature Australian coastal plants and physiological tolerance to exotic woody weed invasion.

3.1 Introduction

Plant growth typically follows diurnal and seasonal patterns which can be quantified by measurements of photosynthesis including stomatal conductance, carbon assimilation, and chlorophyll a fluorescence (e.g. Prior, Eamus and Duff 1997; Karavatas & Manetas 1999; Stylinski, Gamon and Oechel 2002). Such measurements have shown that photosynthesis follows an annual cyclic pattern which is dependent on climatic conditions (Larcher 2003). Additionally, individual plant growth rates can be influenced by microhabitat parameters including nutrient levels, water availability and shading. During periods where conditions are not ideal for growth plants use a range of strategies to cope with different environmental conditions experienced in the short to longer term (Long, Humphries and Falkowski 1994; Bazzaz 1996).

Most studies on seasonal photosynthetic efficiencies have been conducted in the Northern hemisphere on deciduous species and the physiological processes toward winter photosynthetic minimums and subsequent leaf abscission (e.g. Ensminger, Busch and Huner 2006). Studies in Mediterranean and arid regions suggest that evergreen sclerophyllous plants also experience photosynthetic minima during the cold and often dry winter period, even in the absence of frost (Kyparissis, Drilias and Manetas 2000; Larcher 2000). Sclerophyllous evergreen plants of the south-eastern Australian coast experience a temperate environment with maximum autumn and minimum spring rainfall. On average, monthly rainfall has historically been above ca. 70mm which is makes this region much wetter than the Mediterranean which can experience long rainless and cloudless periods

(Kyparissis *et al.* 2000). Here we report on the seasonal photosynthetic efficiencies of three evergreen sclerophyllous species native to the coastal dune systems of south-eastern Australia: *Correa alba* (Rutaceae), *Lomandra longifolia* (Lomandraceae) and *Monotoca elliptica* (Epacridaceae). Fluctuations in photosynthetic capacities are likely to follow seasonal trends of moisture and temperature however these patterns may vary when coupled with disturbances that cause plant stress.

Additionally, we studied whether the seasonal photosynthetic efficiencies of mature individuals of these three species were affected by the invasion of an exotic woody shrub, bitou bush (*Chrysanthemoides monilifera* spp. *rotundata*; Asteraceae). South African bitou bush is known to alter the abiotic conditions of the south-eastern Australian coast by increasing soil moisture, decreasing ground incident radiation, moderating ground temperature extremes and altering the nitrogen cycle (Lindsay & French 2004, 2005). Abiotic parameters such as temperature, irradiance and moisture levels determine the microclimate and drive plant growth and species distribution (Tilman 1988; Austin 1990; Bazzaz 1996). Modification of these parameters outside the typical range can result in extinction of species unless the species has sufficient dispersal abilities to establish in a more favourable environment or has inherent developmental or physiological plasticity to facilitate acclimation to the new conditions (Harper 1977; Bazzaz 1996; Gutschick & BassiriRad 2003).

C. alba, M. elliptica and *L. longifolia* may have the inherent phenotypic or physiological plasticity to cope with the new conditions and demonstrate acclimation or tolerance (Larcher 2003). As the resident species have adapted to the stressful conditions associated with coastal environments, they are likely to possess high physiological plasticity to cope with the range of physiological stresses associated with high salinity, low

water and low nutrient availability, and therefore may be able to tolerate or acclimate to the new conditions induced by invasion (Kozlowski & Pallardy 2002).

Chlorophyll fluorescence is a powerful technique for assessing the photosynthetic health of plants and is widely used by plant ecophysiologists to measure photosynthetic functioning and plant stress (Bolhar-Nordenkampf, Lonig, Baker, Oquist, Schreiber and Lechner 1989; Jones 1992; Bjorkman & Demmig-Adams 1995; Schreiber, Bilger and Neubauer 1995). Fluorescence measurements made under natural light conditions determine the quantum yield of photosystem II (Φ_{PSII}); namely what proportion of absorbed light is being used for photochemical reactions, indicating short term photoinhibition. The light saturated, in situ photosynthetic efficiency (Pmax) is an indicator of overall photosynthetic performance. Measurement of the maximum quantum yield of PSII (F_v/F_m), performed on a dark adapted leaf allows determination of chronic photosynthetic stress (Schreiber *et al.* 1995). Although the photosynthetic ETR calculated from chlorophyll fluorescence cannot be assumed to represent the rate of carbon fixation under field conditions (Maxwell & Johnson 2000), comparison can be made within species and this methodology is very useful when making multiple measurements across seasons and sites. Combined assessment of Φ_{PSII}, Pmax and Fv/Fm enables determination of short and long term photoinhibition as a result of environmental or biotic stressors. Similar parameters have been utilised to demonstrate photoinhibition caused by drought (Lu & Zhang 1998), elevated carbon dioxide (Roden, Egerton and Ball 1999) and insect damage (Stone, Chisholm and Coops 2001).

Assessment and comparison of the mean Fv/Fm of plants between environments provides an assessment of the population level differences. However assessment of the

differences in the variability of Fv/Fm for plants between habitats provides insight into the individual responses to the environment and is useful if we are interested in investigating adaptation, natural selection or acclimation. We proposed that medium and high variability in Fv/Fm is indicative of environmental stress or a heterogeneous environment. Low variability would therefore indicate a homogeneous environment.

The objectives of this study were to map the seasonal photosynthetic patterns of several mature, evergreen, sclerophyll plants of the eastern Australian coast and determine whether the invasion of bitou bush altered the physico-chemical properties of the habitat and subsequently induced photosynthetic stress. Furthermore, we hypothesized that the homogeneous moderated microclimate induced by the bitou bush invasion (Lindsay & French 2004) would result in low variability in photosynthetic efficiencies (Fv/Fm) between plants. Alternatively, the mature resident plants studied may vary in their inherent capacity to cope with the new conditions, such as the change from high light to low light conditions, and therefore an increase in Fv/Fm variability between plants could result.

3.2 Materials and methods

3.2.1 Study location

This study was conducted on the New South Wales coast between Birdie Beach (-33˚11'S 151˚38'E) and Wairo Beach (-35˚40'S 150˚41'E). Six bitou bush (*Chrysanthemoides monilifera* spp. *rotundata* (DC.) T. Norl.) invaded and six non-invaded sites were randomly chosen where the species were present and where there was minimal disturbance. The study area experiences mean annual maximum and minimum temperatures of 23.9˚C and 9.2˚C respectively and annual average rainfall of 1241.0mm at the southern end of the study range (Ulladulla) varying to an annual mean maximum and

minimum temperatures are 25.2˚C and 9.3˚C and annual average rainfall of 1227.5mm in the north (Norah Head) (Australian Bureau of Meteorology 2006). Rainfall is highest in May (late autumn). The study area is characterized by Holocene sand exposed beaches with parallel sand dune systems.

Bitou bush has invaded inland from the fore-dune through to the hind-dune littoral rainforest or coastal woodland communities along the New South Wales coast. Hence the indigenous study species were representative of these two systems: *Correa alba* (Andrews) (Rutaceae), an endemic shrub of the New South Wales sand dunes; *Monotoca elliptica* ((Sm.) R.Br.) (Epacridaceae), a medium sized tree found in the central eastern Australian hind dunes; and a rush, *Lomandra longifolia* (Labill.) (Lomandraceae), which has a wide distribution in eastern Australia.

3.2.2 Microhabitat physico-chemical characteristics

Several physico-chemical parameters were assessed around five individual plants of each species in each of five bitou bush invaded and non-invaded sites. Three soil samples beneath the crown of each individual were taken from ca. 20cm below ground level and pooled to make one sample per individual. Soil pH was determined electronically after adding 10 ml of distilled water to 10g of soil and ammonium-nitrogen, nitrate-nitrogen and plant available phosphorus (Olsen method) analyses were conducted by the Victorian Department of Primary Industries (April 2005). The average of three litter depth measurements below the crown of each individual plant was calculated. Canopy cover for each individual was estimated by the percentage of projected foliage in a 2 m radius area above the foliage of each plant.

In early 2006, we also measured the ground incident temperature and light below the canopy of 17 bitou bush (only) canopies and 17 indigenous species canopies on the fore dune at Corrimal Beach (centre of the study area), which is *C. alba* habitat. Temperature was measured with ibutton® temperature dataloggers (Maxim Dallas Semiconductor) at 20 minute intervals over 27 days (from the 13th of January to the 8th of February 2007).

3.2.3 Seasonal in-situ Φ_{PSII} and Pmax of *C. alba*, *M. elliptica* and *L. longifolia* in invaded and non-invaded habitats

Invaded sites were characterized by having at least 70% bitou bush ground cover. Non-invaded sites were bitou bush free and dominated by intact indigenous vegetation. The instantaneous in-situ electron transport rates (ETR) and leaf incident photosynthetically active radiation (PAR) of five leaves, of five different individuals, at each of six sites in both the invaded and non-invaded habitats were assessed. Measurements were taken non-intrusively in the field using the portable miniaturised Pulse Amplitude Modulated fluorometer (mini PAM, Heinz Walz, Effeltrich, Germany) once in each season: October 2004 (spring), January 2004 (summer) April 2004 (autumn) and August 2005 (winter). Leaves were arbitrarily selected from throughout each plant and were required to be mature, not senescent, void of visible damage, of horizontal orientation to the sun and in the outer most region of the canopy. Measurements were taken before 11am and after 2pm to avoid periods of midday photosynthetic depression and photoinhibition (Larcher 2003). The fibre-optic fluorescence measuring device was kept at a constant distance (ca. 1cm) and angle (ca. 60˚) from the leaf lamina using the mini PAM leaf clip.

3.2.4 Seasonal Fv/Fm of *C. alba, M. elliptica* and *L. longifolia* in invaded and non-invaded habitats

Following measurements of in-situ ETR in each season, five different leaves from each plant were removed and dark-adapted in the shade for 30 minutes. The ratio of variable to maximum chlorophyll fluorescence (Fv/Fm) was measured using the mini PAM. Healthy plants are expected to have an Fv/Fm of approximately 0.83 and a significant decline in this value indicates photoinhibition (Bjorkman & Demmig-Adams 1987).

3.2.5 Statistical analysis

Comparisons between habitats for each physico-chemical parameter were conducted using a mixed General Linear Model (SPSS Version 12.0) with sites nested within habitat. Many of the parameters were log transformed (ln(x+1)) to meet the assumptions of the ANOVA. The Corrimal Beach ground incident light data was fourth root transformed.

For the statistical analysis of the photosynthesis measurements, individual plant data was used rather than leaf level data (see Givinish 1988). Numbers for individual plants were based on the mean of the 5 leaves assessed for each individual in each site, season and habitat. Light response curves of ETR were plotted to determine the regions were light was limiting (Φ_{PSII}) and where light saturated maximum photosynthetic rate was achieved (Pmax). To determine whether there was a difference between the Φ_{PSII} of plants in the invaded and non-invaded habitats, for each season we analysed Φ_{PSII} for plants in sites that experienced PAR of less than 300 $\mu molm^{-2}s^{-1}$ for *C. alba*, less than 250 $\mu molm^{-2}s^{-1}$ for *M. elliptica* and PAR less than 200 $\mu molm^{-2}s^{-1}$ for *L. longifolia.* These values were chosen as

this was the linear region between PAR and ETR before the light compensation point (light limited region). An ANCOVA was used to explore differences in Φ_{PSII} between habitats and sites nested within habitats using PAR as a covariate. Pmax was similarly assessed using an ANCOVA by comparing the ETR for plants in sites that experienced PAR greater than 700 $\mu molm^{-2}s^{-1}$ for *C. alba*, 550$\mu molm^{-2}s^{-1}$ for *M. elliptica* and greater than 650 $\mu molm^{-2}s^{-1}$ for *L. longifolia* as this was where ETR began to flatten indicating that maximum level of photosynthesis had been achieved. Parameters were ln (x+1) transformed to meet the assumptions of ANOVA.

To determine whether there were differences between species, habitats and seasons in Fv/Fm we conducted a 3 factor ANOVA with sites nested within habitats (SPSS Version 12.0). Significant differences between seasons were determined using the Student-Neumann-Keuls (SNK) multiple comparison test. We also assessed whether there were significant differences in the variability of plant-level Fv/Fm between and within sites in the invaded habitat compared to the non-invaded habitat using the F test (Zar 1999).

3.3 Results

3.3.1 Climate

Although the start of the study period experienced good conditions there was tendency for a decline in rainfall accompanied by an increase in temperatures during the sampling period relative to the long term averages (Fig. 3.1). The rainfall during 2005 was lower than the long term averages for both the southern (Ulladulla) and northern (Norah Head) ends of the study site however the 2004 spring experienced very high monthly rainfall (Figs 3.1a and 3.1d). The maximum temperatures during the sampling period were

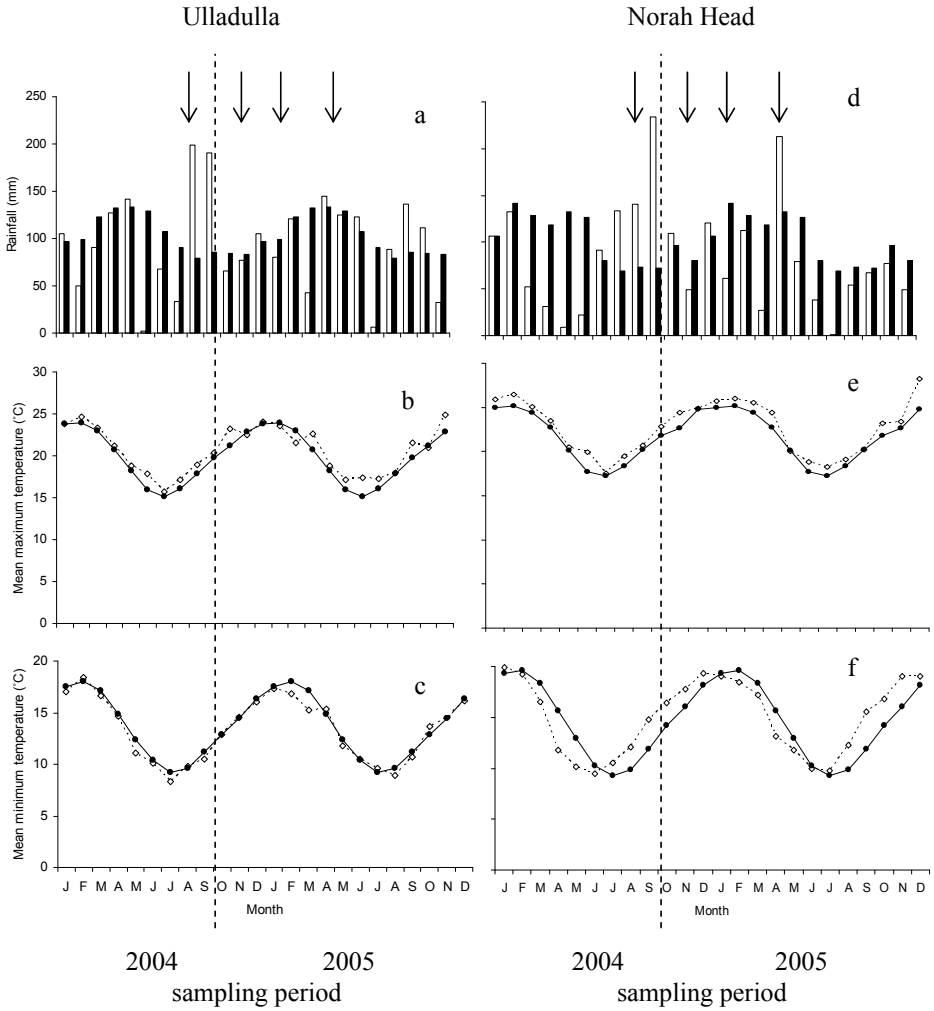


Figure 3.1: Monthly rainfall (a and d) and monthly mean daily maximum (b and e) and minimum (c and f) temperatures at the Southern end (Ulladulla) and Northern end (Norah Head) of the study range during 2004 and 2005 (broken line). The long term monthly averages (unbroken line) are from 94 years at Jervis Bay (near Ulladulla) and the last 30 years at Norah Head. Arrows show sampling dates.

generally 1-2˚C higher than the long term averages however the southern end of the study site experienced slightly lower mean maximum monthly temperatures during autumn (Fig. 3.1b). The mean monthly minimum temperatures were slightly above the long term averages during the early stages of sampling (spring) however they dropped below the long term averages at both ends of the study site during summer and autumn (Figs 3.1c and 3.1f).

3.3.2 Microhabitat physico-chemical characteristics

The pH, litter depth, nitrates, ammonium and plant available phosphorus levels were significantly different between sites irrespective of habitat for all species resulting in no overall difference between the invaded and non-invaded habitats (Table 3.1; Fig. 3.2). We did however detect that the canopy cover above *C. alba* was significantly greater in the invaded compared to the non-invaded habitat ($F_{1,8}$=36.689, p<0.001, Table 3.1; Fig. 3.3). Conversely, there was an overall lower canopy cover above *M. elliptica* in the invaded habitat compared to the non-invaded habitat ($F_{1,8}$=36.689, p<0.001, Table 3.1; Fig. 3.3).

Parameter	*C. alba*				*M. elliptica*				*L. longifolia*			
	Site(habitat)		habitat		Site(habitat)		habitat		Site(habitat)		habitat	
	$F_{8,40}$	p	$F_{1,8}$	p	$F_{8,40}$	p	$F_{1,8}$	p	$F_{8,40}$	p	$F_{1,8}$	p
Ammonium	3.61	0.003**	0.05	0.83	4.69	<0.001***	0.68	0.43	3.56	0.003**	4.12	0.08
Nitrates	4.86	<0.001***	1.28	0.29	2.50	0.03*	1.06	0.34	12.77	<0.001***	0.99	0.35
Phosphorus	10.68	<0.001***	2.44	0.16	9.41	<0.001***	3.19	0.11	11.77	<0.001***	4.73	0.06
pH	104.55	<0.001***	0.68	0.43	26.78	<0.001***	2.16	0.18	29.42	<0.001***	2.86	0.13
litter depth	3.18	0.007**	2.57	0.15	4.29	0.001***	0.26	0.63	4.99	<0.001***	0.54	0.48
canopy	1.97	0.08	36.69	<0.001***	5.07	<0.001***	5.42	0.048*	23.17	<0.001***	0.49	0.51

Table 3.1: Comparison of various physico-chemical parameters of *C. alba, M. elliptica* and *L. longifolia* between habitats, and sites within habitats (site (habitat)).

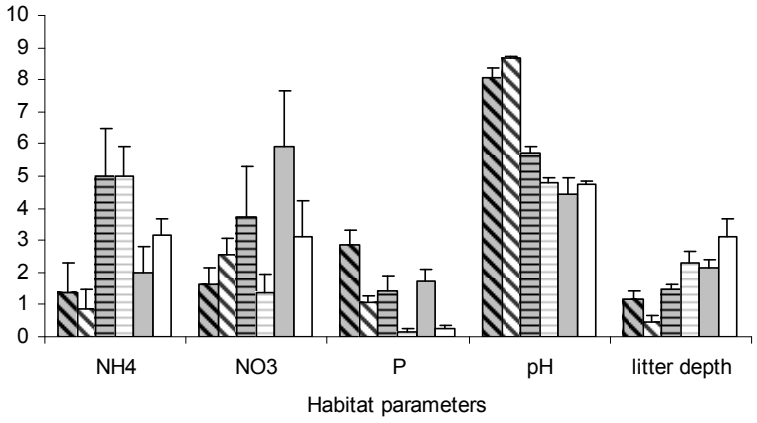


Figure 3.2: Mean NH4 (mg/kg), NO3 (mg/kg), P (mg/kg), pH (pH units) and litter depth (cm) associated with *C. alba* (diagonal pattern), *M. elliptica* (horizontal pattern) and *L. longifolia* (no pattern) in the invaded (grey bars) and non-invaded (white bars) habitats. Error bars represent one standard error.

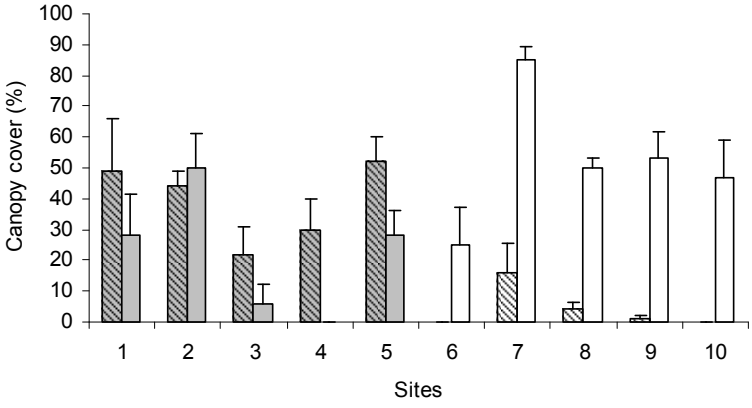


Figure 3.3: Mean percentage canopy cover above *C. alba* (diagonal pattern) and *M. elliptica* (no pattern) in the invaded (grey bars) and non-invaded sites (white bars). Errors bars represent one standard error.

On the fore dune at Corrimal Beach, the ground incident light was significantly reduced beneath bitou bush (mean: 11.96 μmolm^{-2}s^{-1}, SE: 3.26) canopies to 1.5% of the radiation found beneath non-invaded canopies (mean: 821.12 μmolm^{-2}s^{-1}, SE: 111.906) ($F_{1,48}$=209.951; P<0.001; Fig. 3.4). The ground level temperatures below the bitou bush canopy during summer were also significantly milder with a significant reduction in the maximum daily temperatures (invaded mean: 26.2˚C, SE: 0.2˚C; non-invaded mean: 45.6˚C, SE: 0.5˚C; $F_{1,917}$=1041.916; P<0.001) and increase in the daily minimum temperature (invaded mean: 22.2˚C , SE: 0.2˚C; non-invaded mean: 17.5˚C, SE: 0.1˚C; $F_{1,917}$=36.997; P<0.001) compared to below the native canopies (Fig. 3.5). These changes reduced the daily range from 28 ˚C in non-invaded habitats to 4 ˚C in invaded habitats.

Figure 3.4: Mean ground incident light in the invaded (grey bar) and non-invaded (white bar) habitats at Corrimal beach. Error bars represent one standard error.

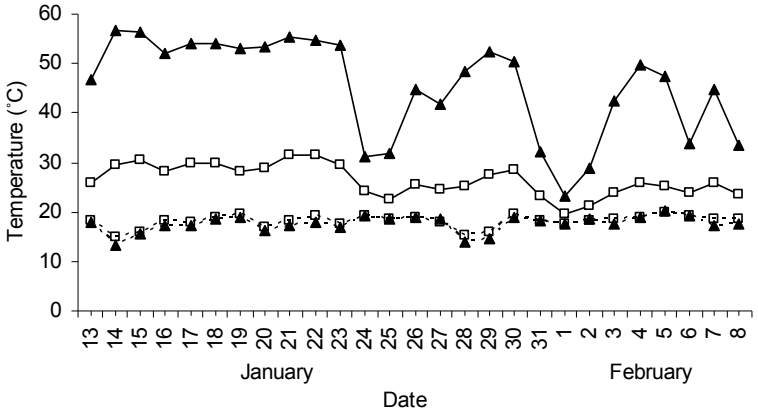


Figure 3.5: Daily maximum (solid line) and minimum (broken line) ground level temperatures under the invaded canopy (open square) and the non-invaded (closed triangle) canopy during early 2007.

3.3.2 Seasonal in-situ Φ_{PSII} and Pmax of mature *C. alba, M. elliptica* and *L. longifolia*

We detected no overall effect of season or habitat on the Φ_{PSII} of any of the three species studied (Table 3.2). Small differences in these factors were largely compounded by variation at the site level resulting in significant interaction terms. The Φ_{PSII} of *C. alba* was significantly influenced PAR ($F_{1,29}$=18.02; P<0.001) and by the interaction between sites (nested within habitat) and seasons ($F_{1,29}$=9.95; P<0.001), sites (nested within habitats) and PAR ($F_{8,29}$=8.33; P<0.001), PAR and seasons ($F_{3,29}$=3.84; P=0.019) and between PAR and habitat ($F_{1,29}$=13.55; P=0.001). Similarly, the Φ_{PSII} of *M. elliptica* was significantly affected by PAR ($F_{1,46}$=40.36; P<0.001) and by the interaction between sites (nested within habitat) and seasons ($F_{10,46}$=10.70; P<0.001), sites (nested within habitats) and PAR ($F_{9,46}$=2.12; P=0.047), and between PAR and seasons ($F_{3,46}$=5.15; P=0.004). Additionally, the Φ_{PSII} of

M. elliptica was also significantly affected by the interaction between sites (nested within habitat), season and PAR ($F_{1,46}$=9.88; P<0.001) suggesting that individuals of this species have highly variable responses under different light regimes at different sites in different seasons. Conversely, the Φ_{PSII} of *L .longifolia* was not significantly affected by any of the factors studied.

		Invaded				Non-invaded			
		Pmax		Φ_{PSII}		Pmax		Φ_{PSII}	
Species	Season	mean	SEM	mean	SE	mean	SEM	mean	SEM
C. alba	Summer	202.97	11.91	35.51	5.36	105.79	7.99	27.31	2.89
	Autumn	167.95	8.29	58.73	11.65	146.76	6.47	34.64	6.65
	Winter	141.33	6.70	47.43	2.97	144.08	8.02	48.80	4.61
	Spring	104.26	4.46	31.15	4.99	110.78	7.85	22.21	3.45
M. elliptica	Summer	74.62	8.08	16.80	2.94	100.07	6.88	25.89	2.84
	Autumn	126.64	10.25	16.34	2.66	100.35	7.17	7.16	1.13
	Winter	92.88	4.98	33.33	3.86	109.92	4.71	33.21	4.80
	Spring	110.60	10.54	17.20	2.55	-	-	19.74	1.58
L. longifolia	Summer	79.16	25.22	16.62	3.45	106.30	6.11	21.46	3.50
	Autumn	154.79	10.46	15.33	5.68	139.76	8.15	5.92	0.11
	Winter	115.47	6.06	36.40	3.20	115.81	9.61	47.79	3.19
	Spring	91.46	-	22.04	1.75	90.56	-	21.00	2.76

Table 3.2: Mean in-situ Pmax and Φ_{PSII} for each species in each season in the invaded and non-invaded habitats. SEM: Standard error of the mean.

The in-situ Pmax of all species was similarly not significantly affected by habitat or season alone. We did however find that the Pmax of *L. longifolia* was affected by the interaction between site (nested within habitat), season and PAR ($F_{4,31}$=3.35; P=0.022) as well as site (nested within habitat) and season ($F_{4,31}$=3.67; P=0.015).

3.3.4 Seasonal Fv/fm of *C. alba, M. elliptica* and *L. longifolia* in invaded and non-invaded habitats

There was a significant difference in Fv/Fm between sites irrespective of habitat, season and species ($F_{42,576}$= 2.948; P < 0.001). We found no overall effect of habitat and no

interaction between habitat and season. However we did find significant differences between species ($F_{2,14}$= 11.302; P = 0.001) and seasons ($F_{3,45}$ = 22.649; P < 0.001). *C. alba, M. elliptica* and *L. longifolia* all displayed significantly different Fv/Fm patterns (*L. longifolia* mean 0.8066; *C. alba* mean 0.8186; *M. elliptica* mean 0.8288). Across all species, the highest Fv/Fm was found in winter (mean 0.839) followed by autumn (mean 0.8224) and the lowest Fv/Fm was found in spring (0.8032) and summer (mean 0.8074) which were statistically similar. For each species SNK tests showed that winter consistently elicited the highest Fv/Fm and summer the lowest, with differences in autumn and spring between species (Figs 3.6 – 3.8).

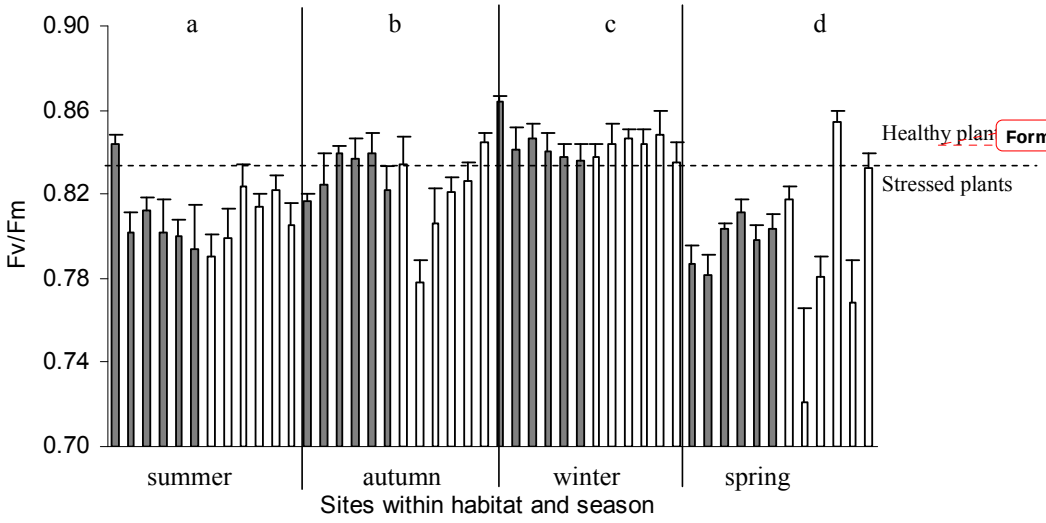


Figure 3.6: Mean Fv/Fm of *C. alba* individuals at each of six sites in invaded (grey bars) and non-invaded (white bars) habitats in each season. Bars represent one Standard Error.

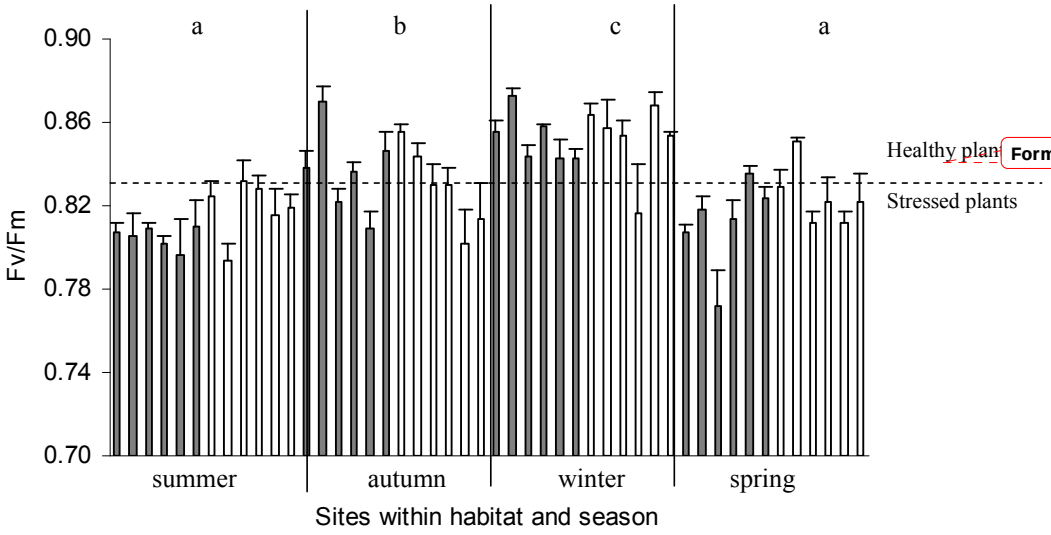


Figure 3.7: Mean Fv/Fm of *M. elliptica* in each site in the non-invaded (white bars) and invaded (grey bars) habitats for each season. Bars represent one Standard Error.

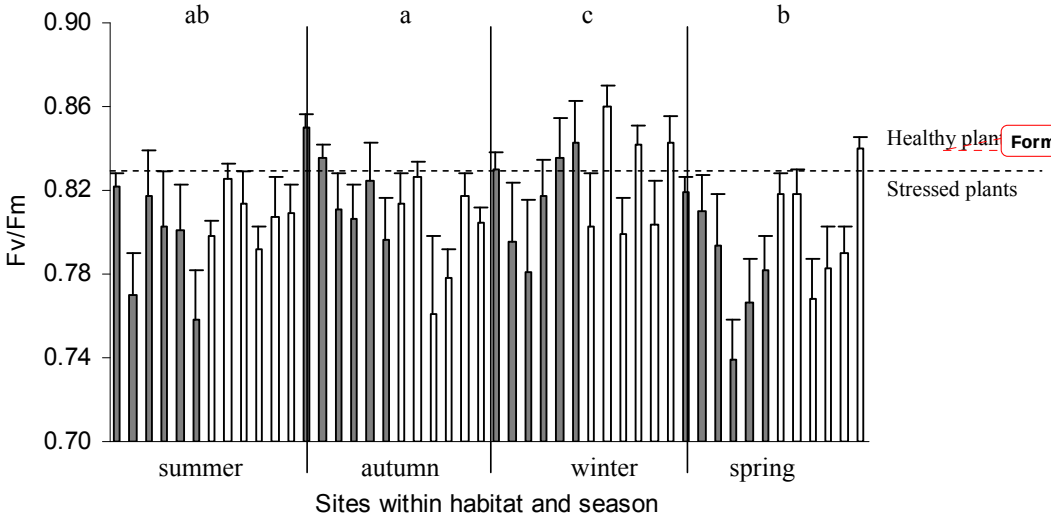


Figure 3.8: Mean Fv/Fm of *L. longifolia* in each site in the non-invaded (white bars) and invaded (grey bars) habitats for each season. Bars represent one Standard Error.

The variability in Fv/Fm within sites revealed that all species in the invaded habitat in autumn had significantly less variability in Fv/Fm compared to those in the non-invaded sites (*C. alba* $F_{24,24}$= 2.18; P = 0.03; *M. elliptica* $F_{24,24}$= 4; P < 0.001; *L. longifolia* $F_{24,24}$= 2.67; P = 0.01) (Fig. 3.9). In the invaded habitat, *M. elliptica* and *C. alba* showed the least amount of variability within sites with the mean square being less than 0.0005 in all seasons except in summer for *C. alba* (Fig. 3.9). In spring in the invaded habitat, *C. alba* showed significantly less variability in Fv/Fm between ($F_{5,5}$= 11; P = 0.01) and within ($F_{24,24}$= 8; P < 0.001) sites compared to conspecifics in the non-invaded habitat (Fig. 3.9).

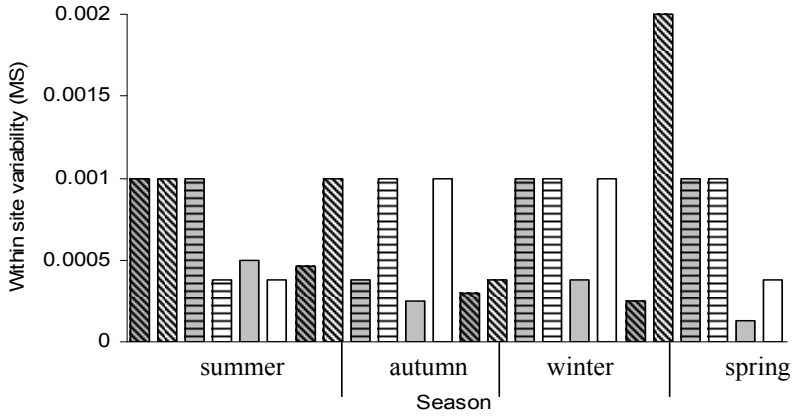


Figure 3.9: The within site variability (mean square, MS) for each *C. alba* (diagonal pattern), *L. longifolia* (horizontal pattern) and *M. elliptica* (no pattern) in the invaded (grey bars) and non-invaded (white bars) habitats in each season.

Significantly less variability in Fv/Fm was also expressed by *M. elliptica* within sites in the invaded habitat in winter compared to the variability found within sites in the non-invaded habitat ($F_{24,24}$= 8; P = 0.03). *L. longifolia* in the invaded and non-invaded habitat showed a comparable amount of variation in Fv/Fm in winter and spring with more

variation in the non-invaded habitat compared to the invaded habitat in autumn ($F_{24,24}$= 2.67; P = 0.01) (Fig. 3.9). Conversely, in the invaded habitat in summer, *L. longifolia* had significantly less variability than conspecifics in the non-invaded habitat ($F_{24,24}$= 2.67; P = 0.01).

3.4 Discussion

In the non-invaded habitat, photosynthesis of all three species was optimal in winter, during and after the maximum annual rainfall season, as indicated by the high Fv/Fm (plant stress indicator). Conversely spring and summer were more stressful and likely to coincide with decreased productivity for all three species in non-invaded habitats. These results emphasize the importance of water availability for growth in these environments and the potentially negative impacts of high summer temperatures and associated drought conditions. Despite these seasonal changes it is also clear that photosynthesis occurs throughout the year and that the substantial down regulation of photosynthesis reported for Mediterranean evergreens (Karavatas & Manetas 1999; Werner, Correia and Beyschlag 2002) is absent in these environments. This is logical if the rainfall and temperature patterns of the eastern Australian coast are considered, which are relatively consistent compared to the extreme fluctuations found in Mediterranean and Northern Europe where most of the seasonal photosynthesis studies have been undertaken. In the present study, *C. alba* exhibited the lowest site mean Fv/Fm of 0.72 in spring which is only a reduction of 15% from the site mean maximum level of photosynthesis recorded for this species. Therefore I suggest that evergreen sclerophyllous species of mesic environments have the capacity to photosynthesise all year round without substantial photoinhibition. This is of course dependent on the level of stomatal opening, irradiance,

leaf water deficit and absence of extreme environmental stress. However the future climate of this area which is predicted to have higher temperatures and more extreme fluctuations in water availability (Hughes 2003) may exaggerate these seasonal differences.

Bitou bush had no significant effect on soil nutrient levels, soil pH or leaf litter depth. However, we did find significant differences in parameters associated with the canopy cover of the invaded habitat (ground level temperature and light), which was differentially altered for the three species studied. The significant decrease in canopy cover on the fore dune associated with *C. alba*, significantly moderated the ground level microclimate by reducing the daily maximum temperatures, increasing the daily minimum and reducing the ground incident light levels. Interestingly, we found that the canopy cover differences had no significant effect on the seasonal photochemical (Φ_{PSII}) or biochemical (Pmax) capacities of these native species, suggesting physiological tolerance to the invasion by mature plants. The Pmax of *C. alba* was twice as high in summer in the invaded compared to the non-invaded habitats, suggesting that the reduced temperature and shading by bitou bush was mitigating the summer extremes for this species. This finding also lends support to our conclusion that low water availability and high temperatures have a more negative impact on photosynthesis in these species than reduced light.

The general lack of a physiological response to the invasion was unexpected, as leaves are typically predicted to acclimate to new light environments through physiological or morphological changes (Sims, Seeman and Luo 1998; Rothstein & Zak 2001; Rozendaal, Hurtado and Poorter 2006). Late successional species and particularly those that are mature evergreen sclerophylls, such as the species of this study, were expected to show physiological changes, as described by Bazzaz (1996). We therefore predicted that the

increased canopy cover above *C. alba* might force these otherwise sun-plants to become shade-plants, and vice-versa for *M. elliptica*. However this was not the case. Mature individuals of all three species appeared to tolerate the new neighbour.

Differences in physico-chemical parameters were more pronounced between sites than between habitats. Similarly, the photosynthetic capacities of the three plants studied were primarily dependent on the location of the plant (site) and the season. The site level differences detected in this study are consistent with ecological theory regarding the patchiness of resources (Gonzalez-Megias, Gomez and Sanchez-Pinero 2007) and the consequent site specificity of plant responses (Bazzaz 1996; Schurr, Walter and Rascher 2006; Warren, Dreyer, Tausz, and Adams 2006). Even though the canopy and microclimate of some resident species was significantly altered by the bitou bush invasion, the endogenous physiological mechanisms of these mature, evergreen sclerophyllous species appeared to be plastic enough to allow tolerance to the new habitat. In fact, it appears that the new conditions induced by the invasion may be more favourable for *C. alba* and *M. elliptica* as the variability in Fv/Fm was significantly lower than those in the non-invaded habitat, particularly in Autumn and Spring. This decrease in variability in Fv/Fm is however potentially cause for concern considering the likelihood of future environmental change. Exposure to environmental heterogeneity is important for the maintenance of genetic variability (Hedrick 1986; Hoffman & Parsons 1997). Alternatively, the reduction in variability could be due to the loss of certain susceptible genotypes from the habitat, an indication of possible genetic divergence (Agrawal 2001). Monitoring of in-situ plant survival following invasion is required to explain the decline in Fv/Fm variability found in this study.

Other studies on invasive woody species have shown that an invader may elicit feedbacks which facilitate the monoculture formation (e.g. Vitousek & Walker 1989; Evans, Rimer, Sperry and Belnap 2001; Klironomos 2002). These studies have focused on the mean population level changes that occur after invasion by assessing species abundance or richness. Rarely has the physiological impact of an invader on mature indigenous species been assessed. Physiological techniques for detection of plant stress or phenological shifts can be important indicators enabling early detection of invasion impacts at different life history stages.

Chapter 4: Determination of potential allelopathy and indirect soil chemical interference by an exotic invasive plant using a comprehensive bioassay protocol

4.1 Introduction

Despite the ease of naturalization of many plants in foreign countries, comparatively few become invasive and form monocultures (Williamson 1996). The mechanisms facilitating the invasion of exotic plants, resulting in the displacement of indigenous flora, are generally accepted to be a combination of resource and interference competition (Williamson 1996; Amarasekare 2002; Inderjit *et al.* 2005). Here we refer to resource competition as the altered outcome for an organism arising from the use of a common resource (Amarasekare 2002) and interference competition as the negative outcome to an interacting organism as a result of the direct or indirect alteration of the environment by the other. Competition for resources is often cited as the driving force behind plant invasions and is intimately linked to enemy release and resource fluctuation hypotheses (Darwin 1859; Crawley 1997; Davis *et al.* 2000). However this mechanism is often adopted without adequate experimentation or exploration for possible underlying interference mechanisms (Levine *et al.* 2003; Schenk 2006; Meiners 2007). For example, reduced nutrient levels of one plant may be due to the faster or superior acquisition of nutrients by another plant (resource competition) or prevention of access to the nutrients by the release of deterrent compounds (interference competition) (de Kroon *et al.* 2003). Interference competition is possibly more influential in low resource environments where plants have evolved resource conservative strategies (Grime 1979). Direct interference via allelopathy (Molisch 1937;

Muller 1966; Rice 1974) or indirect interference via abiotic or biotic modification of plant derived compounds, are less accepted as mechanisms of invasion, although mounting evidence supports the occurrence of these phenomena (Goldberg & Barton 1992; Reigosa *et al.* 1996; Wardle *et al.* 1998; Amarasekare 2002; Pellissier *et al.* 2002; Hierro & Callaway 2003; Inderjit *et al.* 2006).

Historically, arguments against allelopathy as a mechanism of direct interspecific interference are based on methodological inadequacies including insufficient controls and the lack of convincing field studies (Harper 1977). More recently, significant improvements in methodology and technology have facilitated the demonstration of allelochemical exudation (Tang 1986; Bais *et al.* 2003; Inderjit & Nilsen 2003; Walker *et al.* 2003) and the biochemical mechanisms of action (Einhellig 1986; Einhellig *et al.* 1993; Dayan *et al.* 2000; Inderjit & Duke 2003; Duke & Oliva 2004). Allelochemicals are known to be released via decomposition of plant materials, volatile emissions, and exudation (Rice 1984), however to affect the growth and development of neighbouring plants, they must travel from the source plant through either the soil or air. The air and soil comprise environmental filters that are spatially and temporally heterogeneous, containing a plethora of biotic and abiotic elements which can influence the integrity and residence time of the plant-derived compounds (Inderjit 2001, 2005; Tharayil *et al.* 2006). For example, bacterial degradation of the allelopathic compound, juglone has been shown to reduce the effect of allelopathy by black walnuts (*Juglans nigra*) (Williamson & Weidenhamer 1990). The soil has an important influence on plant coexistence due to its heterogeneous, diverse and complex biotic (Paterson *et al.* 2006), abiotic and physical composition (Hodge 2004). Most plant-derived compounds are likely to flow into the soil except volatiles compounds

from shoots and leaves. Hence analysis of the soil chemistry is integral to studies of allelopathy (Inderjit & Weiner 2001).

Plants that have co-evolved are more likely to have developed the capacity to tolerate the environment that their co-evolved neighbours create compared to the environment created by exotic invasive plants (Callaway & Aschehoug 2000; Fitter 2003). This tolerance is evidenced by the diverse mixture of species generally growing side by side in undisturbed tracts of indigenous vegetation. Successful exotic species invasion into undisturbed vegetation and the eventual formation of monocultures suggests that invasion might be facilitated by interference competition (Fitter 2003). Such mechanisms may also facilitate the ability of some indigenous species to dominate communities.

4.1.1 Protocol for determination of phytotoxicity, allelopathy and indirect soil effects

To assess whether chemical interference competition is a mechanism of exotic plant invasion we adopt a stepwise bioassay guided fractionation procedure that incorporates parallel extractions from the leaves, roots and soil of an exotic plant and those from the dominant indigenous plant. This comprehensive protocol aims to differentiate between phytotoxicity, allelopathy and indirect soil effects of an exotic shrub using bioassays. We differentiate between allelopathic and phytotoxic effects as phytotoxic compounds may exist in plant parts but are not exuded or released into the surrounding environment.

Laboratory-based bioassays of root and shoot extracts are useful indicators of plant phytotoxins however the inclusion and comparison of plant and soil extracts is imperative if we are interested in allelopathy and indirect soil chemical effects on indigenous species. Moreover, comparison of the effects of solvent extracts from the exotic plant and soil with

those from the dominant indigenous plant is important to demonstrate whether the exotic uses interference competition as a novel mechanism of invasion and displacement of native plants, or whether interference competition already occurs in the indigenous system. By comparing the effect of plant and soil extracts of the exotic system to those of the indigenous system at a range of concentrations predicted to occur in the field, we overcome some of the criticism that allelopathy studies have attracted in the literature (Williamson & Richardson 1988; Inderjit & Weston 2000; Romeo 2000; Inderjit & Nilsen 2003; Hoagland & Williams 2004).

We investigated measured decreases in germination, root and shoot growth and assessed whether there was a 50% reduction in germination, root or shoot growth of treated seeds compared to control seeds (LC_{50}). Phytotoxicity was suggested if a) there was a significant effect of leaves or root extract and b) the LC_{50} was reached for the root or leaves extract *and* c) there was no significant comparable soil extract effect. Allelopathy was indicated by a) a root or leaf extract effect *and* b) a comparable soil solvent extract having a significant effect *and* c) the LC_{50} being reached for the roots or leaves and soil extracts. If a soil extract elicited a significant effect on a growth parameter (seedling germination or root or shoot length) and reached the LC_{50}, and comparable shoot and root extracts did not elicit a significant effect, then we suggested that the associated plant (bitou bush or acacia) induced an indirect effect on the soil chemistry which in turn affected the seedling growth parameter of the test species (Table 4.1). If there was a statistically significant and LC_{50} effect of the soil extract only, this suggested that both the acacia and bitou bush soils inhibit the seedling growth parameter.

Table 4.1: Protocol for assessing the presence of phytotoxicity, allelopathy and indirect soil effects from plant roots, leaves and soil extracts of native compared to exotic species (E) using the dose response curve (C), a 2-factor ANOVA testing the effects of E, C and C x E and attainment of LC_{50} for ecological relevant concentrations of extracts.

	Indicators			
	Statistically significant factor ($P < 0.05$)			
Mechanism	C	E	C x E	LC_{50}
Phytotoxicity	roots or leaves	roots or leaves	roots or leaves	Exotic and/or native
Allelopathy	roots or leaves and soil	roots or leaves and soil	roots or leaves and soil	Exotic or native
Indirect soil effects	soil only	soil only	soil only	Exotic or native

4.2 Materials and methods

4.2.1 Exotic species

Bitou bush (*Chrysanthemoides monilifera* spp. *rontundata* L.; Asteraceae) is a South African woody shrub which was planted on the sand dunes of the New South Wales (Australia) coast to stabilize the sand dunes following rutile and zircon mining from 1946 to 1964 (Barr 1965; Agriculture and Resource Management Council of Australia & New Zealand *et al.* 2000; Conservation 2006; DEC 2006). However bitou bush subsequently spread into relatively undisturbed tracts of native vegetation, so that in 2000 bitou bush had invaded approximately 80% of the New South Wales coastal sand dune vegetation (Agriculture and Resource Management Council of Australia & New Zealand *et al.* 2000; DEC 2006). Many plant species, populations and communities are currently threatened by the bitou bush invasion which was declared a key threatening process under the New South Wales *Threatened Species Conservation Act* (1995) in 1999. Studies have shown that bitou bush seedlings outcompete seedlings of the native dominant species of the New South

Wales sand dune vegetation, *Acacia longifolia* var. *sophorae* (Labill.) F. (Muell.) (acacia; Fig. 4.1), through faster root growth, greater water uptake and

Figure 4.1: *Acacia longifolia* var. *sophorae* (left) is the dominant indigenous shrub of the eastern Australian foredunes (right).

greater leaf area than the acacia (Weiss & Noble 1984). Further equivocal evidence suggests that bitou bush may be allelopathic as bitou bush litter decreased the germination and seedling growth of acacia and bitou bush soil inhibited seedling growth but not the germination of acacia (Vranjic *et al.* 2000). Copeland (1984) also found undeveloped evidence suggesting bitou bush allelopathy, as the germination and seedling growth of three woody heath species (*Eucalyptus viminalis*, *Hakea dactyloides* and *Casuarina littoralis*) appeared to be differentially inhibited by bitou bush root and shoot water leachates. However this study suffered from fungal attack in the Petri dishes containing the seed bioassays. A third study has also shown that bitou bush leaf litter inhibited the germination of *Hardenbergia comptoniana* and *Lepidium sativum* (cress) and that the water soluble bitou bush leaf extract decreased the germination of *Schoenia filifolia* and *L. sativium* (Hughes 1998). In congruence with these preliminary suggestions of bitou bush allelopathy

we aimed to conduct a comprehensive assessment of different fractions of bitou bush leaves, roots and soil in comparison with similar extracts from the native dominant (Weiss and Noble 1984b) of the invaded system, *A. longifolia* var. *sophorae* against five native species and a universal test species, *Lactuca sativa* (lettuce; Asteraceae).

4.2.2 Bioassay test species

Five endemic species of the bitou bush invaded region of the New South Wales coast were selected: *Acacia longifolia* var. *sophorae* (woody shrub; Fabaceae); *Banksia integrifolia* (tree; Myrtaceae); *Actites megalocarpa* (herb; Asteraceae); *Lomandra longifolia* (rush; Lomandraceae); and *Isolepis nodosa* (sedge; Cyperaceae). Utilisation of taxonomically and morphologically distinct species facilitated generalization of results. Additionally, we employed *Lactuca sativa* as a universal indicator of phytotoxicity (see Escudero *et al.* 2000; Iqbal *et al.* 2002). The lettuce seed was purchased from a commercial supplier (Mrs. Fothergills's, "All season" lettuce) and the native seeds were collected and pooled from at least five different sites along the New South Wales south coast from Moruya (35˚91'S 150˚15'N) to Kurnell (34˚0'S 151˚21'N).

4.2.3 Extraction procedure

Fresh bitou bush and acacia roots (500 g), leaves (500 g) and soil (2 kg) from 10-20 cm beneath at least five plants of each species (within 10 cm from live, visible roots) were collected from North Wollongong, New South Wales, Australia in July 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 fresh leaf and root (lightly brushed to remove soil) material

was chopped with scissors and the soil sample was sifted to remove all biological material. The raw materials were placed into separate conical flasks and dichloromethane (DCM; HPLC grade)) (1 L for roots and leaves; 2 L for soil) was added. After 30 hours the DCM was decanted from each flask (supernatant) and replaced sequentially with acetone (AR grade), methanol (AR grade) and distilled water (all in equal volumes as used for the DCM extraction) in 30 hour cycles. After removal of the supernatant and before adding the next solvent, each solvent was evaporated under reduced pressure from a water bath (temperature < 40 ˚C) (Büchi Rotavapor). The resultant residues are hereafter referred to as the solvent extracts. DCM extracts alkaloids, aglycones and volatile oils; acetone extracts alkaloids, aglycones and glycosides; methanol extracts glycosides and sugars; and water extracts glycosides, sugars and amino acids (Houghton & Raman 1998).

4.2.4 Extract concentrations

The effect of each solvent extract on seedling growth was assessed by utilizing the dose response of six concentrations: 0, 10, 100, 500, 1000 and 2000 ppm (parts of solvent extract/ million parts of solvent (distilled water)). These concentrations were based on the concentrations (w/w; weight of extract/ weight of original soil used) of various bitou bush and acacia DCM soil extracts which were approximately 100-900 ppm. The soil samples were taken in June during the peak flowering period of bitou bush and the peak vegetative growth period of acacia. To incorporate the probable temporal and spatial variation in concentrations of soil allelochemicals we tested a range of concentrations.

4.2.5 Bioassay procedure

For application in the Petri dish bioassays, the methanol and water extracts were readily re-dissolved in distilled water (2 ml). The DCM and acetone extracts were first dissolved in DCM (1 ml) and added to each Petri dish fitted with filter paper. The DCM was then allowed to evaporate from the filter paper (15 mins) before distilled water (2 ml) was added to each Petri dish. Four replicate bioassays of each extract at each concentration were conducted with 20 equidistant seeds set in glass Petri dishes (8 cm diameter). The pH of all Petri dish solutions was recorded using an electronic pH meter (Activon model 209). Controls comprised 20 seeds grown in Petri dishes fitted with filter paper (Whatman number 1) and distilled water (2 ml). Four control Petri dishes were conducted for each of the four bioassay replicates. The response of all species to DCM controls compared to the water controls was also tested. The DCM controls consisted of 20 seeds in each of four replicate Petri dishes fitted with filter paper to which DCM (2 ml) had been applied then evaporated from (15 mins), followed by the addition of distilled water (2 ml).

Replicates were conducted through time in an incubator (Fig. 4.1) set to a diurnal (12 hr/12 hr) temperature (15/25 ˚C) and light regime. After 7, 23, 40, 48, 53 and 59 days for lettuce, *I. nodosa*, *B. integrifolia, A. longifolia* var. *sophorae, A. megalocarpa* and *L. longifolia* respectively, germination and seedling shoot and root length (Fig. 4.1) were recorded.

Figure 4.2: The bioassays were run in an incubator (left) and seedling shoot and root lengths were measured (right).

4.2.6 Statistical analysis

Probit analysis (SPSS Version 13.0) was used to determine whether increasing concentrations (covariate) of comparable extracts of the exotic and native species (factors) differed in effect on germination of each test species. We used Pearson's goodness of fit test to ascertain whether the regression models adequately fit the data. A Z score was used to investigate whether the slopes differed from zero and a parallelism test was conducted to determine whether the slopes of the relationship between germination and concentration of each extract were similar. If the two slopes were not parallel we analysed whether the relationship between germination and concentration was significant for each extract separately.

A two factor ANCOVA (SPSS Version 13.0) was conducted to assess whether the root and shoot length of any of the test species elicited different responses to the bitou bush and acacia extracts (Extract), there was a significant dose response when both extracts were

combined (Concentration) or whether there was a different response to different extract species at different concentrations (Extract x Concentration). Extract species was a fixed factor and concentration was a covariate in the model. Data was ln(x+1) transformed to satisfy data normality and variance homogeneity if these assumptions of the ANOVA were violated.

4.3 Results

4.3.1 Effects of bitou bush and acacia extracts

The bitou bush roots and soil had a slightly higher percentage weight of hydrophobic (DCM and acetone soluble) compounds than the acacia which had more polar (methanol and water soluble) material (Fig. 4.2). The opposite was found for the acacia leaves which had a higher percentage weight of DCM soluble compounds to the bitou bush which had a greater percentage weight of acetone, methanol and water soluble compounds (Fig. 4.2). The proportion of each solvent extract of the soil was similar for the two species, except bitou bush had a slightly higher percentage weight of hydrophobic compounds.

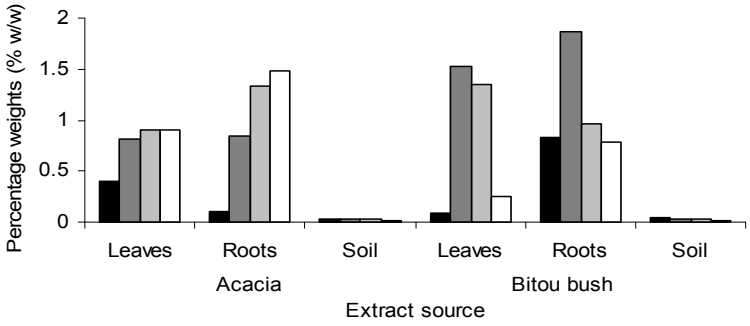


Figure 4.2: Percentage weights (w/w) of the DCM (black bars), acetone (dark grey bars), methanol (light grey bars) and water (white bars) solvent extracts of the leaves, roots and soil of the acacia and bitou bush.

The pH of the methanol and acetone extracts of the acacia shoots and roots and the pH of the acacia soil methanol extract significantly decreased with concentration (Table 4.2). For bitou bush extracts, only the methanol extract of the shoots showed a significant decrease in pH with increasing concentrations (Table 4.2). At 2000ppm, the highest mean pH (7.27) was demonstrated by the DCM extract of the bitou bush soil, and lowest mean pH (5.00) was demonstrated by the acacia leaf acetone extract (Table 4.2).

Table 4.2: Mean pH range of extract concentrations (10 to 2000ppm) and the significance values of an ANOVA testing whether the pH differed with extract concentrations. * P<0.05, **P<0.01.

Extract species	Plant part	Solvent extract	$F_{(4,15)}$	P	Mean pH range (10-2000ppm)
Acacia	leaves	DCM	0.75	0.574	7.23-7.05
		acetone	3.41	0.036*	5.78-5.00
		methanol	4.25	0.017*	6.34-5.21
		water	0.56	0.698	6.44-6.48
	roots	DCM	0.91	0.486	7.20-6.97
		acetone	4.95	0.010*	6.97-5.46
		methanol	4.61	0.013*	6.92-5.49
		water	0.74	0.580	6.53-6.18
	soil	DCM	0.45	0.772	7.24-7.24
		acetone	0.45	0.077	7.11-6.35
		methanol	5.91	0.005**	6.60-5.22
		water	0.54	0.706	6.33-6.33
Bitou bush	leaves	DCM	0.86	0.508	7.28-6.83
		acetone	2.89	0.059	6.34-6.01
		methanol	3.55	0.031*	6.61-5.33
		water	0.60	0.670	6.98-6.38
	roots	DCM	1.71	0.804	6.74-7.17
		acetone	0.40	0.201	6.65-6.08
		methanol	1.56	0.235	6.56-5.18
		water	0.96	0.457	6.09-6.69
	soil	DCM	0.35	0.838	7.07-7.27
		acetone	1.65	0.215	7.68-6.76
		methanol	0.82	0.533	6.86-6.20
		water	1.91	0.161	6.16-7.07

To test whether applying the hydrophobic extracts to the Petri dish/filter paper with DCM had a confounding effect on seedling growth, we determined whether lettuce

seedlings grown on DCM evaporated filter paper differed in length to those grown on regular filter paper. We found no significant effect of filter paper type on lettuce germination ($F_{(1,6)}$=0.43 P=0.537), shoot ($F_{(1,6)}$=0.83; P=0.431) or root ($F_{(1,6)}$=0.07; P=0.804) length.

4.3.2 Effects on germination

High unexplained variability in germination resulted in significant deviations in most of the Goodness of fit tests, indicating that the models were did not tightly fit the data (analyses not presented). Despite this high variability, regression coefficients and tests of differences in slopes between extract species yielded significant differences indicating that while only a small proportion of the variability is explained by the treatments, it is nevertheless a predictable component.

A significant effect on the germination of at least one of the test species was found for most of the bitou bush leaf extracts, none of the acacia leaf extracts, and all of the root extracts from both the acacia and bitou bush (Table 4.3). Although, no extract had an effect across a broad range of species, the DCM extract of the bitou bush root was most inhibitory to the species studied (Table 4.3). Furthermore, the bitou bush root extracts (acetone and water) exhibited allelopathic affects against the germination of three of the test species (Table 4.3). The hydrophobic extracts of the bitou bush and acacia soils also significantly affected the germination of *B. integrifolia* and *L. longifolia* respectively.

Table 4.3: Coefficients, parallelism tests and goodness of fit of the probit regression comparing the relationship between increasing concentrations of extracts from each extract

source species (acacia and bitou bush) and the germination success of 6 species. Values in bold are significant at $\alpha = 0.05$.

Extract		Bioassay Species	Regression coefficient and Z score		Regression parallelism test (df=1)		Z score for each regression		LC_{50}
Plant part	Solvent		Coefficient ± SE ($x10^{-5}$)	Z	χ^2	P	acacia	bitou	
Leaves	DCM	*L. sativa*	**-53±11**	**-4.67**	0.00	1.000			
		A. longifolia	**-12±6**	**-2.14**	0.27	0.604			
		B. integrifolia	-2±6	-0.40	**9.78**	**0.002**	**-2.63**	**2.10**	bitou
		A. megalocarpa	-31±6	-5.08	**11.29**	**0.001**	-1.24	-5.91	
		L. longifolia	-11±7	-1.96	0.14	0.713			
		I. nodosa	**14±7**	**2.08**	1.47	0.226			
	acetone	*L. sativa*	-28±16	-1.79	1.00	0.317			
		A. longifolia	**28±6**	**4.78**	2.00	0.157			
		B. integrifolia	**-14±6**	**-2.47**	0.10	0.758			
		A. megalocarpa	13±7	1.89	0.50	0.480			
		L. longifolia	**25±6**	**3.79**	**7.68**	**0.006**	1.74	**3.76**	
		I. nodosa	**-14±6**	**-2.18**	0.38	0.537			
	methanol	*L. sativa*	4±18	0.24	0.09	0.769			
		A. longifolia	**-15±6**	**-2.65**	3.43	0.064			
		B. integrifolia	**-19±6**	**-3.40**	0.04	0.84			
		A. megalocarpa	-6±6	-1.00	**5.83**	**0.016**	1.17	-2.61	
		L. longifolia	-9±6	-1.58	**4.38**	**0.036**	0.56	**-2.79**	bitou
		I. nodosa	-3±6	-0.45	2.81	0.094			
	water	*L. sativa*	-8±14	-0.59	1.45	0.228			
		A. longifolia	5±6	0.88	**22.70**	**<0.001**	**4.22**	**-2.89**	bitou
		B. integrifolia	8±6	1.26	**8.94**	**0.003**	**2.74**	-0.85	
		A. megalocarpa	-11±6	-1.82	**5.21**	**0.022**	-3.17	0.65	
		L. longifolia	**24±6**	**4.13**	0.00	1.00			
		I. nodosa	5±6	0.81	2.98	0.084			
Roots	DCM	*L. sativa*	18±19	0.93	0.00	1.000			
		A. longifolia	**-16±6**	**-2.89**	2.24	0.134			bitou, acacia
		B. integrifolia	**-13±6**	**-2.35**	0.01	0.704			
		A. megalocarpa	**-21±6**	**-3.50**	1.78	0.182			bitou, acacia
		L. longifolia	-6±6	-1.11	**5.66**	**0.017**	1.06	**-2.59**	bitou
		I. nodosa	11±6	1.83	3.08	0.079			
	acetone	*L. sativa*	-1±14	-0.58	1.43	0.232			
		A. longifolia	**-14±6**	**-2.52**	**6.65**	**0.01**	0.23	**-3.78**	bitou
		B. integrifolia	**-41±6**	**-7.11**	**10.46**	**0.001**	**-7.24**	**-2.75**	acacia
		A. megalocarpa	-1±6	-0.23	1.43	0.232			
		L. longifolia	-11±6	-1.95	0.135	0.713			
		I. nodosa	-7±6	-1.15	**5.46**	**0.020**	**-2.05**	0.56	
	methanol	*L. sativa*	-25±13	-1.92	**7.64**	**0.006**	**-2.52**	0.59	
		A. longifolia	**-24±6**	**-4.20**	**5.07**	**0.024**	-1.21	**-4.70**	bitou
		B. integrifolia	-6±6	-1.11	0.01	0.917			
		A. megalocarpa	**-41±6**	**-6.52**	1.29	0.257			
		L. longifolia	-6±6	-1.13	1.17	0.280			
		I. nodosa	**-17±6**	**-2.77**	0.00	1.000			
	water	*L. sativa*	-7±14	-0.50	**4.03**	**0.048**	0.75	-0.97	
		A. longifolia	**-23±6**	**-3.97**	**7.76**	**0.005**	**-4.95**	-0.62	acacia
		B. integrifolia	-7±6	-1.20	0.73	0.392			
		A. megalocarpa	-3±6	-0.54	**16.57**	**<0.001**	**2.54**	**-3.16**	bitou

		L. longifolia	2±6	0.42	**3.57**	**0.021**	**2.39**	-1.77	bitou
		I. nodosa	-12±6	-1.94	**25.75**	**<0.001**	**-3.55**	0.79	
Soil	DCM	*L. sativa*	12±27	0.44	1.20	0.273			
		A. longifolia	-7±6	-1.17	0.10	0.751			bitou, acacia
		B. integrifolia	**-16±6**	**-2.74**	0.09	0.760			bitou
		A. megalocarpa	**-25±6**	**-4.16**	0.32	0.570			bitou, acacia
		L. longifolia	-6±6	-1.09	**37.14**	**<0.001**	**-6.55**	0.2	acacia
		I. nodosa	-4±6	-0.64	0.17	0.677			
	acetone	*L. sativa*	-6±18	-0.34	**6.22**	**0.013**	0.59	-0.97	
		A. longifolia	**-13±6**	**-2.28**	0.30	0.584			bitou
		B. integrifolia	-7±6	-1.20	0.73	0.392			
		A. megalocarpa	-5±6	-0.82	1.31	0.252			
		L. longifolia	**-26±6**	**-4.57**	**21.88**	**<0.001**	**-5.24**	**3.79**	acacia
		I. nodosa	-4±6	-0.61	0.81	0.368			
	methanol	*L. sativa*	-12±16	-0.71	**13.16**	**<0.001**	-1.49	0.38	
		A. longifolia	1±6	0.16	0.00	1.000			
		B. integrifolia	**-30±6**	**-5.26**	**5.42**	**0.020**	0.54	0.26	
		A. megalocarpa	**-22±6**	**-3.56**	0.71	0.398			
		L. longifolia	7±6	1.26	**16.42**	**<0.001**	**3.36**	-1.44	
		I. nodosa	**23±7**	**3.27**	**6.90**	**0.009**	**3.61**	0.89	
	water	*L. sativa*	**-50±18**	**-2.76**	2.96	0.085			
		A. longifolia	**-17±6**	**-3.00**	0.165	0.684			
		B. integrifolia	**3±6**	**0.57**	0.04	0.839			
		A. megalocarpa	**-14±6**	**-2.28**	0.07	0.787			bitou
		L. longifolia	-10±6	-1.68	0.017	0.895			bitou
		I. nodosa	-12±7	-1.72	0.01	0.940			

4.3.3 Effects on shoot and root length

All of the leaf extracts from both species inhibited the growth of at least one of the test species. Approximately half acacia and bitou bush leaf extracts were inhibitory to the same species, however, this effect was not seen in the comparable soil extracts, suggesting the effects are from chemicals within leaves that are not released into the soil. The hydrophobic root and soil extracts were more inhibitory than the hydrophilic or more polar extracts and more species were affected by the bitou bush extracts than comparable acacia extracts (Table 4.4).

Table 4.4: Probability values from an ANOVA testing the effect of extract species (E), concentration (C) and the interaction between extract species and concentration (E x C) on

seedling shoot and root length of six species for each solvent extract of each plant part. Values in bold are significantly different at $\alpha = 0.05$. Influential species from post hoc analyses and occurrence of LC_{50} in dose response curves (*) are also shown.

Extract		Bioassay Species	Effects on shoot length			Effects on root length			Influential extract species	
			E	C	E x C	E	C	E x C	shoot	root
Leaves	DCM	*L. sativa*	**0.042**	0.110	0.502	**<0.001**	**0.008**	**0.018**	b	a
		A. longifolia	0.189	**0.016**	0.946	0.613	0.374	0.444	b, a*	
		B. integrifolia	0.618	0.066	0.264	0.376	**0.032**	0.229		b, a
		A. megalocarpa	0.880	0.462	0.742	0.421	0.564	0.541		
		L. longifolia	0.303	**0.019**	0.386	0.713	**0.001**	0.632	b*, a*	b*, a*
		I. nodosa	0.388	**0.037**	0.644	**0.046**	**<0.001**	0.174	b*, a*	b*, a*
	acetone	*L. sativa*	0.593	0.947	0.989	**0.007**	**0.001**	0.478		a
		A. longifolia	0.515	0.163	**0.033**	0.741	0.571	0.278	b, a	
		B. integrifolia	0.224	0.661	0.252	0.062	0.867	0.816		
		A. megalocarpa	0.729	0.549	0.532	0.059	**0.010**	0.554		
		L. longifolia	**<0.001**	0.182	**0.004**	**<0.001**	0.063	**0.003**	a*	a*
		I. nodosa	0.600	0.080	0.408	**0.009**	**<0.001**	0.174		b*, a*
	methanol	*L. sativa*	0.356	0.480	0.701	0.136	0.087	0.909		
		A. longifolia	0.448	0.142	0.331	0.686	0.552	0.802		
		B. integrifolia	0.596	0.831	0.825	0.405	0.282	0.285		
		A. megalocarpa	0.226	0.466	0.305	0.057	**0.019**	**0.019**		
		L. longifolia	0.187	0.357	0.350	**0.001**	0.435	0.199		a*
		I. nodosa	0.990	0.161	0.125	**0.001**	**<0.001**	0.180		b*, a
	water	*L. sativa*	0.665	0.137	0.610	0.532	0.458	0.489		
		A. longifolia	0.273	0.997	0.141	0.526	0.439	0.266		
		B. integrifolia	0.822	**0.047**	0.978	0.824	0.678	0.904		
		A. megalocarpa	0.548	0.741	0.760	0.375	0.536	0.681		
		L. longifolia	0.211	**0.002**	**0.005**	**0.029**	**0.002**	**0.035**	b*, a	b*, a*
		I. nodosa	0.947	0.097	0.542	0.302	0.235	0.689		
Roots	DCM	*L. sativa*	0.077	**0.016**	0.643	**0.002**	0.167	0.366	b*	a*
		A. longifolia	**0.050**	0.933	0.122	0.143	0.990	0.224	b*	
		B integrifolia	0.783	0.087	0.444	0.441	0.102	0.092		
		A. megalocarpa	0.465	**0.015**	0.310	0.234	0.922	0.125	b*	
		L. longifolia	**0.014**	**<0.001**	0.104	0.526	**<0.001**	0.628	b*, a	b*, a
		I. nodosa	0.115	**<0.001**	0.200	**0.047**	**<0.001**	0.374	b*, a*	b*, a*
	acetone	*L. sativa*	0.573	0.609	0.494	**<0.001**	0.460	**0.015**		a
		A. longifolia	0.486	0.462	0.294	0.894	0.895	0.968		
		B. integrifolia	**0.050**	**<0.001**	0.067	0.141	**<0.001**	0.782	b*, a*	b*,a*
		A. megalocarpa	0.533	0.210	0.899	0.920	0.762	0.509		
		L. longifolia	**0.041**	0.096	0.165	0.318	0.105	0.475		
		I. nodosa	0.278	0.139	0.792	**0.042**	**<0.001**	0.357		b*, a*
	methanol	*L. sativa*	0.261	0.410	0.868	**0.021**	0.775	0.133		a*
		A. longifolia	0.493	0.133	0.200	0.248	0.192	0.315		
		B. integrifolia	0.552	0.881	0.976	0.936	0.511	0.998		
		A. megalocarpa	**0.038**	0.422	0.488	0.694	0.706	0.451	b	
		L. longifolia	0.515	0.057	0.222	0.619	0.266	0.096		
		I. nodosa	0.961	0.529	0.963	**0.006**	**0.001**	**0.038**		b*
	water	*L. sativa*	0.465	0.872	0.820	0.140	0.997	0.696		
		A. longifolia	0.151	**0.012**	0.320	0.456	0.259	0.890	b*, a*	
		B. integrifolia	**0.029**	0.741	0.320	0.856	**0.032**	0.620	b*	b, a
		A. megalocarpa	0.639	0.687	0.770	0.858	0.712	0.168		
		L. longifolia	0.257	0.448	0.706	0.334	0.475	0.449		

		I. nodosa	0.529	0.122	0.958	**0.003**	0.439	0.532		
Soil	DCM	*L. sativa*	0.986	0.829	0.238	0.493	0.554	0.136		
		A. longifolia	0.916	0.659	0.994	0.775	0.597	0.816		
		B. integrifolia	**0.029**	**0.047**	**0.044**	0.059	**0.028**	0.450	b*	b*, a*
		A. megalocarpa	0.208	**0.035**	0.505	0.489	0.712	0.204	b*	
		L. longifolia	0.173	**0.040**	0.101	0.461	0.810	0.038	b, a	b*
		I. nodosa	0.368	0.088	0.979	0.647	**<0.001**	0.789		b*, a*
	acetone	*L. sativa*	0.113	0.786	0.747	0.222	0.937	0.925		
		A. longifolia	**0.009**	0.760	0.851	0.198	0.600	0.560	b*	
		B. integrifolia	0.617	**<0.001**	0.066	**0.013**	**0.023**	0.084	b, a	b*
		A. megalocarpa	0.808	0.130	0.300	0.344	0.678	0.905		
		L. longifolia	0.311	0.191	0.159	0.226	0.090	0.073		
		I. nodosa	0.336	0.702	0.795	0.065	0.734	0.599		
	methanol	*L. sativa*	0.708	0.407	0.871	0.448	0.387	0.987		
		A. longifolia	0.528	0.530	0.329	0.661	0.783	0.951		
		B. integrifolia	0.909	0.209	0.503	0.954	**0.036**	0.217		
		A. megalocarpa	0.596	0.487	0.506	0.074	0.623	0.430		
		L. longifolia	0.054	**0.016**	0.691	0.896	**0.004**	0.824	a	
		I. nodosa	0.774	0.729	0.521	0.060	0.939	0.816		
	water	*L. sativa*	0.655	0.221	0.614	0.406	0.852	0.934		
		A. longifolia	0.123	0.757	0.396	0.475	0.769	0.434		
		B. integrifolia	0.592	0.407	0.481	0.443	**0.008**	0.301		
		A. megalocarpa	0.875	**0.043**	0.410	0.060	0.210	0.308	b	
		L. longifolia	0.333	0.486	0.251	0.180	0.640	0.534		
		I. nodosa	0.130	**0.001**	0.750	0.186	0.942	0.752		

4.3.5 Phytotoxic, allelopathic and indirect soil effects

From the germination and seedling growth bioassay results, each extract from the bitou bush and acacia had a phytotoxic effect on at least one of the test species (Table 4.5). Overall, the bitou bush extracts were more phytotoxic, allelopathic and had more indirect negative soil effects than the acacia extracts (Table 4.5). Furthermore, the hydrophobic bitou bush root and soil extracts (DCM and acetone soluble) appeared to demonstrate allelopathy and indirect soil effects on seedling growth of all native test species (Table 4.5).

Table 4.5: Summary of inhibition by extract phytotoxicty, allelopathy or indirect soil effects (+ denotes stimulatory effect) on the test species. A = *A. longifolia* var. *sophorae*; Ac = *A. megalocarpa*; B = *B. integrifolia*; I = *I. nodosa*; L = *L. longifolia*; Le = *L. sativa*.

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

5.4 Discussion

By comparing the effects of hydrophilic to hydrophobic extracts of an exotic invasive plant leaves, roots and soil with comparable extracts from the dominant indigenous shrub against five indigenous species, we have found evidence to suggest that although both indigenous and exotic species have the potential to inhibit the establishment of other species via allelopathy and negative indirect soil chemical effects, exotic bitou

bush affected a broader range of species, including the dominant native acacia (*A. longifolia* var. *sophorae*) which may confer bitou bush a greater competitive advantage against this dominant indigenous shrub and facilitate the invasion, and eventual monoculture formation, of bitou bush. The comprehensive bioassay scheme comparing the biological effects of different plant parts and soil extracts, of an exotic invasive plant and the indigenous dominant species allows inferences as to whether chemical interference competition is likely to occur between these species in the field. Inclusion of soil extracts (Inderjit 2001; Inderjit & Weiner 2001) and exotic versus indigenous comparisons is imperative to this end. This is the first documented research, to our knowledge, that incorporated all of these factors into a bioassay based investigation into potential exotic plant allelopathy.

This study of the chemical interference between plants endemic to low resource environments found that hydrophobic compounds are likely to influence community composition and species dominance. The hydrophobic extracts of both the indigenous acacia and exotic bitou bush were the most inhibitory to all indigenous test species. Hydrophobic compounds such as plant waxes, fatty acids, oils, sterols, terpenes and high molecular weight alkanes are likely to occur in the leaves (Yokouchi 1991), roots (Pomilio *et al.* 2000) and vegetated soil (Franco *et al.* 2000; Chefetz *et al.* 2002; Lin *et al.* 2007). Not only do some hydrophobic compounds have the ability to regulate plant establishment (Langenheim 1994; Angelini *et al.* 2003; Barney *et al.* 2005; Nishida *et al.* 2005), but they are also known to have antimicrobial properties (Deans 1991; Karamanoli 2002; Scher *et al.* 2004) which has ramifications for plant growth, particularly in low resource environments where plant-microbe mutualisms are common (Ernst 1985; Logan *et al.* 1989; Abe & Ishikawa 1999).

The acacia roots and soil were found to inhibit the seedling growth of *I. nodosa* and the acacia soil alone inhibited the growth of *B. integrifolia* and *L. longifolia*. The pH of the hydrophobic (DCM and acetone soluble) acacia root and soil extracts did not alter with increasing concentration of extract which suggests that other characteristics of the constituent compounds were responsible for the observed inhibition of growth rather than the pH. We did not find any inhibitory effects of comparable extracts of the acacia leaves and soil, however, decomposing *Acacia* spp. leaves have demonstrated plant growth inhibition (Gonzalez *et al.* 1995; Bernhard-Reversat 1999). Gas chromatography - mass spectrometry (GC-MS) studies have shown that hydrophobic extracts of acacia roots and soil have similar chemical profiles containing largely a high molecular weight alkane series (C19-33), phenolic compounds, plant sterols and a low concentration of terpenes (Chapter 4). High concentrations of alkanes in the soil from both acacia roots and those derived from leaf waxes are likely to induce water repellency especially in the sandy soils (Franco *et al.* 2000; Roper 2005) where this acacia grows, which is likely to affect seedling growth via reduced soil water availability. Phenolic compounds are recognized plant (Gross 1975; Williams & Hoagland 1982) and microbial (Hattenschwiler & Vitousek 2000; Souto *et al.* 2000) growth regulators and are likely to be primarily responsible for the inhibition of *I. nodosa, B. integrifolia* and *L. longifolia* by acacia roots and soil in this study, and potentially in the field. The presence of phenolic compounds in situ may have further ecological ramifications in relation to their potential effects on nutrient cycling and decomposition via direct effects on the microbial community (Hattenschwiler & Vitousek 2000). Therefore, it appears that direct or indirect interference competition is likely to occur between co-evolved species on the New South Wales coastal dunes. Further growth trials in the field are required to confirm the ecological relevance of the present laboratory studies.

The hydrophobic (DCM and acetone) extracts of bitou bush roots and soil had significant inhibitory effects on *A. megalocarpa*, *B. integrifolia, L. longifolia* and *I. nodosa* establishment. Again, we detected no change in the pH of increasing concentrations of bioactive root and soil extracts, suggesting that pH was not responsible for the observed seedling growth inhibition. GC-MS analyses revealed that bitou bush roots and soil both contained high concentrations of terpenes, particularly sesquiterpenes (Ens upubl. data). Sesquiterpenes are also exuded by *Pinus* spp. roots (Lin *et al.* 2007) and include documented allelopathic (Fischer 1986; Cumanda & Marinoni 1991), antimicrobial (Melin & Krupa 1971; Melcher *et al.* 2003; Scher *et al.* 2004) and herbivore deterrent (Theis & Lerdau 2003) compounds.

The inhibitory and stimulatory effect of some of the soil derived solvent extracts was not evidenced by comparable solvent extracts from a plant part. The activity of the soil extracts alone may be due to either the accumulation of plant derived compounds in the soil, or the indirect modification (biotic or abiotic) of plant derived compounds or by plant alteration of the microbial community which subsequently lead to changes in the soil chemistry. The encapsulation of these indirect soil chemical effects is one of the advantages of comparing both soil and plant based extracts on a range of target species. The residual soil effects captured in the present bioassays are also likely to prevent the re-establishment of native plants after bitou bush removal. A regeneration lag time (of approximately 6 months) following bitou bush control has been observed (Andresen pers. comm.) and is suggested prior to replanting with native stock. Alternatively, fire could be used to speed up the volatilisation of the putative hydrophobic allelochemicals found in this study.

Based on this comprehensive bioassay approach, we suggest that chemical interference between co-evolved species may occur and also be a mechanism of exotic

plant invasion. Bitou bush root and soil extracts were more inhibitory to a broader range of species, including the indigenous dominant acacia, which is likely to lead to bitou bush dominance of this vegetation community. This finding is reciprocated in the field where bitou bush monocultures occur along 400km of the NSW coast (Thomas & Leys 2002).

Chapter 5: Identification of volatile compounds released by roots of an invasive plant, bitou bush (*Chrysanthemoides monilifera* spp. *rotundata*), and their potential biological role

5.1 Introduction

Plant roots release organic compounds into the rhizosphere via decomposition, root cell sloughing, mucilage secretion and exudation (Whipps 1990; Einhellig 1995; Kuzyakov and Domanski 2000). 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 neighboring 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; Inderjit *et al*. 2006). Rhizodeposits move through the soil or enter the atmosphere at different rates depending on the specific 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 polymerize 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 at this time (Walker *et al.* 2003).

This study focused on a comparison of the hydrophobic, volatile components of exotic and native plant roots and soil. Volatile emissions and components of plant roots are of increasing interest as allelopathic (Kong *et al.* 2002; Barney *et al.* 2005; 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 in 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 (McGhie and Posner 1981; Franco *et al.* 2000) 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 also well known for their roles in the prevention of desiccation and chemical defense in leaves (Post-Beittenmiller 1996; Bargel *et al.* 2006). 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 problematic.

Different species exhibit unique chemical profiles which have the potential to create unique chemical microhabitats (Osbourn *et al.* 2003; Field *et al.* 2006). Some species will tolerate or benefit from the chemical environment induced by their neighbor or predecessor, however some species will not. The chemical influence (whether beneficial or inhibitory) of one plant on the growth and development of another is referred to as allelopathy (Molisch 1937). Allelopathy may result from the direct influence of compounds released from the donor plant, or indirectly by compound transformation or alteration facilitated via another environmental property (abiotic or biotic) (Inderjit 2001; Inderjit and Nilsen 2003). For example Blum *et al.* (1993) demonstrated that methionine in the soil increased the

inhibitory effect of *p*-coumaric acid in root exudates on morning glory (*Ipomoea hederacea*) biomass.

More recently, allelopathy has been shown to facilitate the invasion of some exotic species into previously diverse systems (Hierro and Callaway 2003). The resident species of the new host range have not undergone co-evolution with the exotic species and may lack the ability to tolerate the chemical environment created by the new neighbor, particularly if novel bioactive compounds are present (Fitter 2003; Hierro and Callaway 2003). For example Callaway and Aschehoug (2000) found that *Centaurea diffusa* had little effect on its co-evolved Eurasian neighbours but induced strong negative effects on new neighbors in North America. 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 (Williamson 1990; Wardle *et al.* 1998; Mallik, 2002; Blair *et al.* 2005; Blair *et al.* 2006). 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 (Hierro and Callaway 2003; Field *et al.* 2006; Mitchell *et al.* 2006). Field experiments and long-term bioassays have been proposed to incorporate the probable pulses of allelochemical release (Weidenhamer 1996), and field and pot experiments to overcome the confounding effects of resource competition and likelihood of allelochemical synergism, antagonism and modification by microbes and soil components (Weidenhamer 1996; Inderjit and Weston 2000). The strength of allelopathic interactions is dependent on the abiotic and biotic context (Daehler 2003) and an array of studies from the cellular to ecosystem level is often demanded to unequivocally demonstrate allelopathy. Hence the

deployment of bioassays to demonstrate allelopathy has been criticized in the literature (Weidenhamer 1996; Inderjit and Weston 2000), 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 (Inderjit and Weston 2000; 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 bitou bush (*Chrysanthemoides monilifera* spp. *rotundata* (DC.) T. Norl.) invasion of the eastern Australian coast as a case study.

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 1948-1964 to stabilize the sand dunes, particularly following sand mining (Weiss 1986; Agriculture and Resource Management Council of Australia & New Zealand *et al.* 2000). However, by 2000, bitou bush had invaded approximately 80% of the New South Wales coastline, including un-mined areas, and formed monocultures if left unmanaged (Weiss *et*

al. 1998; Agriculture and Resource Management Council of Australia & New Zealand *et al.* 2000). 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 2006). 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 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, due to methodological ambiguities, further investigation is warranted. My other investigations into the bioactivity of hydrophobic (dichloromethane and acetone soluble) to hydrophilic (methanol and water soluble) extracts of bitou bush roots, leaves and soil, suggested that generally, the hydrophobic fraction of the bitou bush root was consistently inhibitory to the germination and seedling root and shoot length of a range of native plants (Chapter 4). I therefore aimed to further explore the allelopathic potential of the hydrophobic extracts of bitou bush in the following series of laboratory

studies, by comparison with extracts from the dominant native shrub in the pre-invaded system, acacia (*Acacia longifolia* var. *sophorae*).

5.2 Materials and methods

5.2.1 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 hr 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).

5.2.2 Soil collection and extraction

Soil from below the canopy of at least five bitou bush plants (soil mass 7220 g) and five acacia plants (soil mass 5980 g) was collected. 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 (2mm 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.

5.2.3 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 Varian 3700 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 x 0.25 mm) (SGE Australia) in the split mode 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 mins. 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).

5.2.4 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 5-10ml 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 (λ 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.

5.2.5 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. Seeds were collected from within the Wollongong area. Four replicates in Petri dishes of each of four concentrations (10, 100, 500, 1000 ppm) for each fraction were prepared. Concentrations were based on the weight of the hydrophobic (DCM soluble) extract of the bitou bush and acacia soil (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 minutes 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.

5.2.6 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).

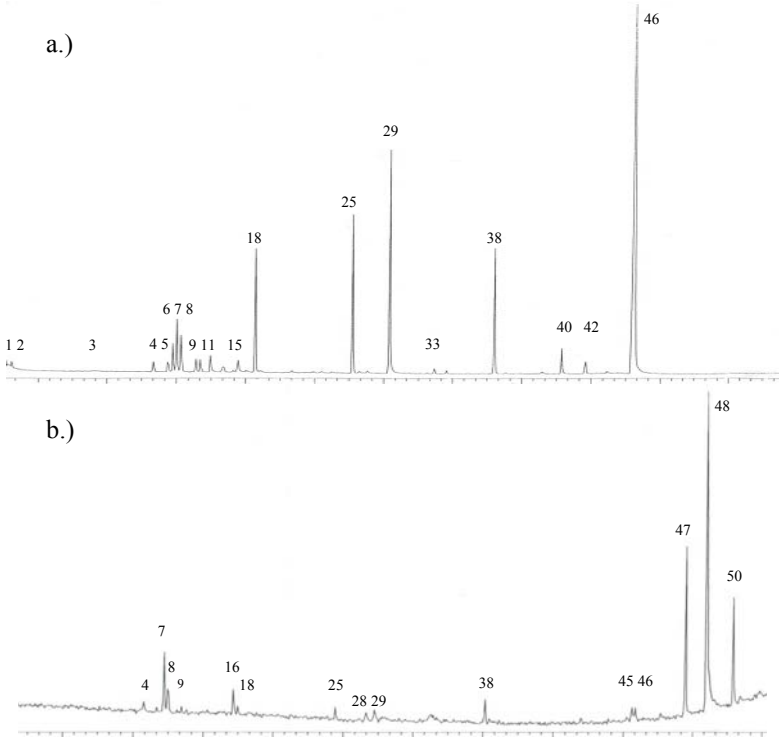
5.3 Results

5.3.1 GC-MS of bitou bush root hydrophobic extract

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 were (0.5 g; 0.1%) and soil (2.06 g; 0.03%) were soluble in DCM. Subsequent GC-MS analysis revealed that the extracts consisted primarily of mono-, sesqui- and diterpenes, phenolic compounds, alkenes and alkanes (Figure 5.1; Table 5.1, 5.2). 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 5.1). Of the compounds detected in the bitou bush soil, only the hexadecanol derivative was unique; while three compounds were also found in the bitou bush root (*β*-isocomene, 7-epi-silphiperfol-5-ene and manool) (Table 5.2). 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, 4b, 8, 8-tetramethyl-3, 4, 10a(1*H*)-phenanthrenetriol , 3-acetate) (Table 5.2). Nine compounds were unique to the acacia root extract (Table 5.2) 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 5.2.

Table 5.1: 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)	-



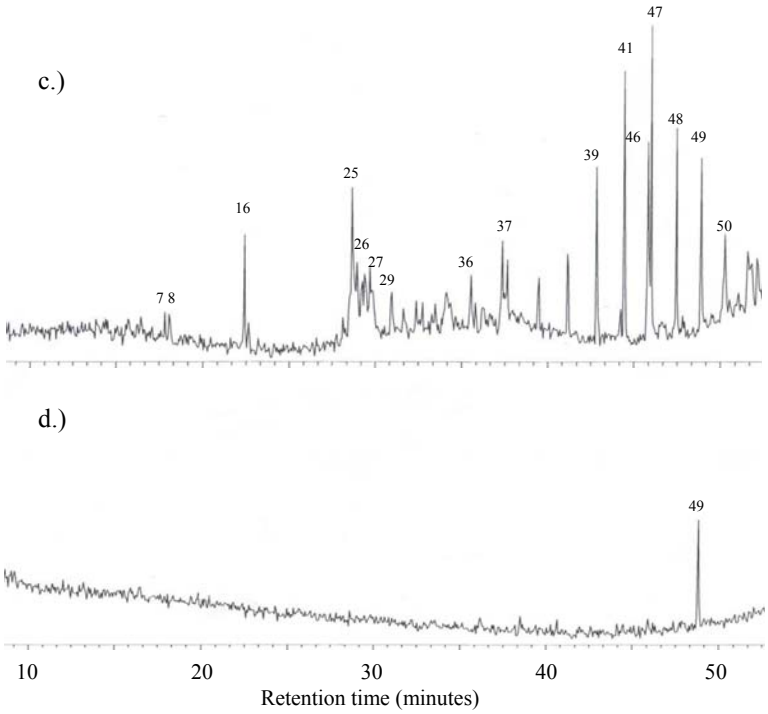


Figure 5.1: Gas chromatograms of the hydrophobic extracts of a.) bitou bush root b.) bitou bush soil c.) acacia root and d.) acacia soil. Numbered peaks are annotated in Table 5.2.

Table 5.2: Components of the bitou bush and acacia root and soil hydrophobic extracts.

						RA[e] (%) of hydrophobic extract components		
No.	Compound	MW[a]	RT[b]	RI[c]	KI[d]	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	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	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	2.43	-
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
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	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

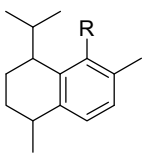
[a] MW, Molecular weight from GC-MS data

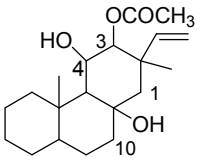
[b] RT, experimental Retention Time (mins) determined on a BP5 column using a homologous series of *n*-alkanes

[c] RI, experimental Retention Index, experimental

[d] KI, Kovats Index

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





30 R = OH

26 R = OCH_3

46

Figure 5.2. Primary constituents of the hydrophobic bitou bush root extract. Compound numbers refer to those in Table 5.2.

5.3.2 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 fraction 2 and 1, which both contained numerous compounds on the basis of GC-MS analysis (Tables 5.3 and 5.4).

Table 5.3: 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

Fraction 1 contained 15 compounds with the major components being pentadecene (41.09%), 5-methoxycalamenene (**26**) (20.34%) and pimaradiene (12.18%). Fraction 2 contained 20 compounds, with major components including 5-methoxycalamenene (**26**) (46.12%), manool (22.90%) and *δ*-cadinene (8.10%). Fraction 3 largely contained manool (92.04%). Fractions 4 and 5 both contained primarily 5-hydroxycalamenene (**30**) (74.80% and 86.08% respectively) and abietol (23.58% and 8.03% respectively). Fraction 7 contained an unidentified sterol and the phenanthrenetriol derivative (**40**).

Table 5.4: GC-MS detection of compounds in each column fraction of the bitou bush root hydrophobic extract. Compounds greater than 1% relative abundance (RA) are shown, except for those that were unique to the bitou bush invaded soil.

Column fraction	Compound	RA (%)
1	*β*-maaliene	2.66
	α-isocomene	1.97
	β-isocomene	0.90
	allo-aromadendrene	1.97
	γ-muurolene	1.32
	pentadecene	41.09
	α-muurolene	2.69
	δ-cadinene	1.25
	cadala-1(10)3,8 triene	5.37
	caryophyllene oxide	1.43
	5-methoxycalamenene (**26**)	20.34
	pimaradiene	12.18
	sandaracopimaradiene	6.09
2	7-epi-silphiperfol-5-ene	0.20
	α-copaene	1.31
	β-maaliene	2.66
	α-isocomene	2.22
	β-isocomene	1.00
	cymene	4.20
	allo-aromadendrene	1.00
	pentadecene	4.19
	δ-cadinene	8.10
	5-methoxycalamenene (**26**)	46.12
	pimaradiene	1.78
	sandaracopimaradiene	1.14
	manool	22.90
3	5-methoxycalamenene (**26**)	5.31
	sandaracopimaradiene	1.77

	manool	92.04
4	5-hydroxycalmenene (**30**)	74.80
	abietol	23.58
5	caryophyllene oxide	2.56
	5-methoxycalamenene (**26**)	2.42
	5-hydroxycalamenene (**30**)	86.08
	abietol	8.30
6	a sterol	8.85
	abietol	47.79
	a sterol	43.36
7	a sterol	30.0
	2-ethenyldodecahydro-2, 4b, 8, 8-tetramethyl-3, 4, 10a(1*H*)-phenanthrenetriol , 3-acetate (**40**)	70.0

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

5.3.3 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 (Figure 5.3). 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, 7 (Table 5.5 and Figure 5.3).

Table 5.5: 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. Significance level α=0.05.

		Germination		Shoot length		Root length	
	df	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 x C	14	0.99	0.477	6.07	<0.001	4.26	<0.001

The root and shoot length of *I. nodosa* were differentially affected by different column fractions at different concentrations (Table 5.5). 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. 5.2). At 500ppm, fractions 4, 1 and 2 reduced *I. nodosa* shoot length to approximately 30%, 50% and 60% (respectively) of the water control (Fig. 5.2). 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. 5.2). The *I. nodosa* roots and shoot lengths were significantly more affected by the higher concentrations (500ppm and 1000ppm) compared to the lower concentrations, suggesting an inhibition threshold at 500ppm. There were no significant differences in the pH at each concentration for each fraction (Table5.6).

Therefore, the primarily low molecular weight GC-volatile terpenes of fractions 1 and 2 and the phenolic compounds contained in fraction 4 (Table 5.6) at 500ppm appeared to be most inhibitory to the growth of *I. nodosa.*

Table 5.6: 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.

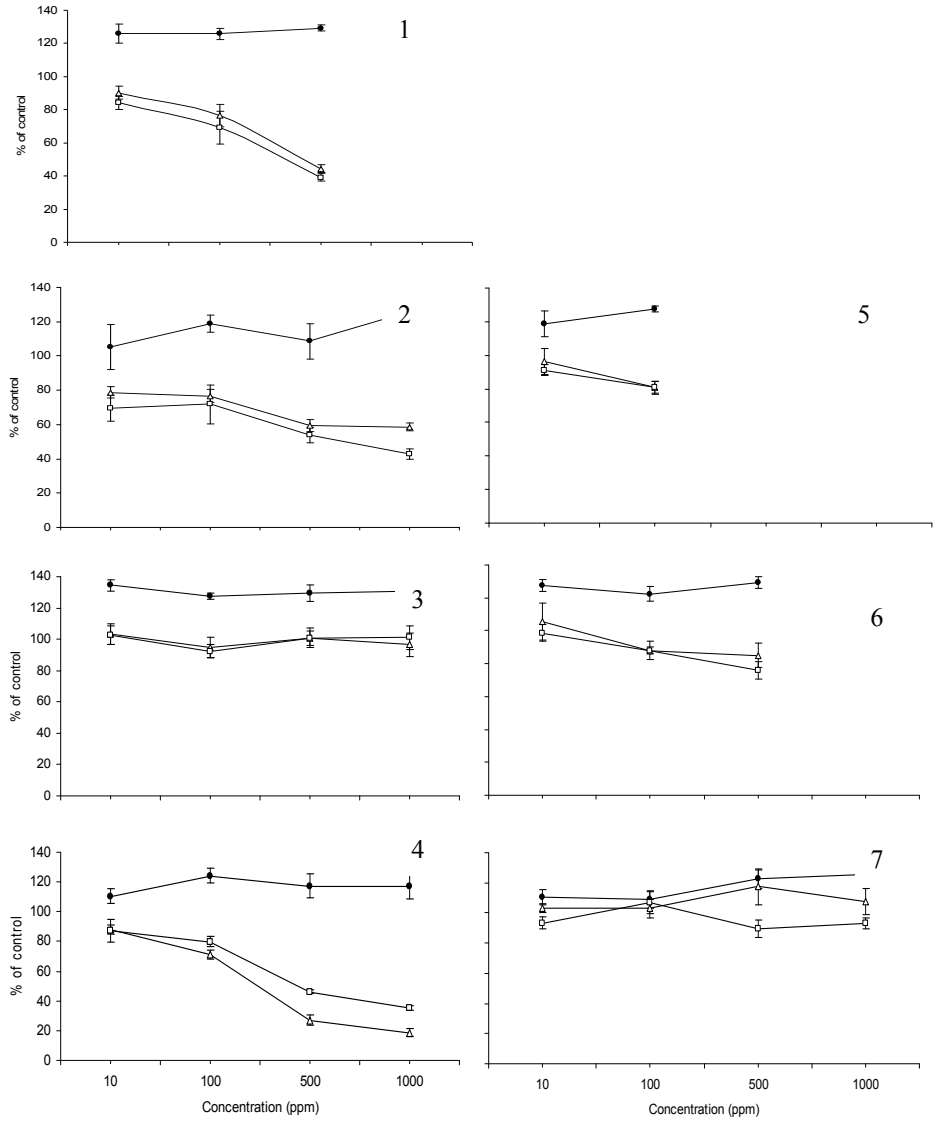


Figure 5.3: Mean dose response curves of *I. nodosa* to each column fraction (1 to 7) of the hydrophobic bitou bush root extract. Closed circles indicate the germination response, open triangles the shoot length and open squares the root length expressed as a percentage of the control after 23 days of incubation. Error bars represent one standard error.

5.4 Discussion

The chemical profile of the bitou bush invaded root-soil system was distinct 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. I have therefore shown that this exotic woody weed is likely to change 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 I suggest that South African bitou bush is allelopathic in the Australian environment. This evidence complements a previous study which found that the shoot and root biomass of *Acacia longifolia* var. *sophorae* was significantly lower when grown in bitou bush soil compared to acacia soil and that bitou bush litter significantly reduced the germination success of *A. longifolia* var. *sophorae* (Vranjic 2000).

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 (20.94%) 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, attraction of symbiotic organisms and pollinators, nutrient cycling (White 1994) and allelopathy (Fischer 1994; Duke 2004).

The chemical profile of the acacia root hydrophobic extract was characterized by the presence of the C_{19}-C_{32} alkane series. To my 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 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). Secondly, the production of alkanes may play a role in habitat 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 2006) and ecosystem function.

Six compounds were common to the bitou bush and acacia roots however we detected marked differences in their presence within the respective soils. These six compounds were present in the bitou bush soil but absent in the acacia soil. Furthermore, nine 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 dunes (Weiss 1984), 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 1984), there is likely to be an increased concentration of root exudates, root cell sloughing and root turnover (Iijima 2003) and therefore a greater release of compounds into the soil, enabling better detection.

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 ten compounds unique to the bitou bush root-soil with the most notable being the 5-hydroxycalamenene (**30**) which made up 94.8% 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 2004). Antimicrobial activity of 5-hydroxycalamenene was also found as a function of the wound protection compounds exuded by *Tilia* spp. (Melcher 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 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*. I have found no other documented evidence for the biological activity of this compound. The phenanthrenetriol derivative (**40**) constituted the greatest proportion of the hydrophobic bitou bush root extract and most of fraction 7, however I 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 1979).

There is thus 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 were shown to be phytotoxic against a native sedge in this study and may have antimicrobial activity as suggested by other researchers.

Chapter 6: Detection of soil chemical interference competition: a novel and rapid technique

6.1 Introduction

Empirical evidence demonstrating interspecific chemical interference competition between plants, or allelopathy, has accrued over the last few decades in line with the popularity of invasion biology. However broad acceptance of the concept of allelopathy has been hampered by past methodological inadequacies and poor ecological extrapolation of laboratory-based studies (Inderjit & Callaway 2003). Hence, methods are continually being improved to facilitate unambiguous detection. For example, continuous trapping methods of allelochemicals from roots (Tang & Young 1982) and leaves (Barney *et al.* 2005), and experimental designs that distinguish between resource and interference competition (Nilsson 1994; Weidenhamer 1996), have been developed.

All plant-derived compounds, except for leaf volatiles, enter the soil matrix where they may be biotically (Huang *et al.* 1999; Inderjit 2001) or abiotically (Inderjit 2005) modified. Plant derived compounds include those that are actively exuded or passively diffuse from living plants, or are leached from decaying plant materials (Waller & Feng 1996). Additionally, plant-derived compounds may also indirectly alter the soil chemistry via alteration of the microbial community (Hattenschwiler & Vitousek 2000). Hence the soil is crucial to studies of allelopathy (Inderjit 2001; Inderjit & Weiner 2001)

It is hypothesised that chemical interference would be more likely to occur between plants that have not co-evolved (Callaway & Aschehoug 2000; Rausher 2001) as a result of differing historical selection pressures. However, interspecific competition between

indigenous species is also suspected to be ubiquitous in nature (Amarasekare 2002) and may influence species composition. This study aimed to contribute to the understanding of soil chemical interference between plants, or allelopathy, using a novel and rapid adsorption technique. I investigated the soil chemical profile of soil invaded with an exotic shrub, bitou bush (*Chrysanthemoides monilifera* spp. *rotundata* (L.) T. Norl.; Asteraceae); soil inhabited by indigenous dominant shrub, acacia (*Acacia longifolia* var. *rotundata* Labill.; Fabaceae); and soil not supporting any vegetation (bare sand). Hydrophobic compounds were specifically targeted based on previous studies showing that hydrophobic solvent derived extracts of bitou bush roots and soil inhibited a range of indigenous species and were more inhibitory than hydrophilic extracts (Chapter 4). Soil hydrophobic compounds were trapped in situ using adsorbent resin filled bags, then extracted from the resin and applied to seedling growth bioassays using an indigenous sedge. I used resin specifically designed to adsorb hydrophobic compounds, although different types of resin could be used to adsorb different general chemical classes. As I was interested in detecting potential hydrophobic, non-polar allelochemicals, I used GC-MS for compound indentification.

6.2 Materials and methods

6.2.1 Resin bags

Seventy five small calico bags (15 cm x 5 cm) were each filled with 10 g of Amberlite® XAD4 industrial grade polymeric resin (Rohm Hass Co.) (Fig. 6.1). The filled bags were thoroughly washed in distilled water, twice in dichloromethane (DCM; HPLC grade), dried and stored in an air-tight glass jar prior to use. Use of plastic utensils was avoided to prevent contamination by plasticizers.

Figure 6.1: Photograph of calico, resin-filled bags.

6.2.2 Study site

The study was conducted on the fore dune at Corrimal Beach, Corrimal, NSW, Australia, where the extant indigenous vegetation was dominated by coastal acacia (*Acacia longifolia* var. *sophorae*) and *Spinifex sericea* towards the strandline. South African bitou bush (*Chrysanthemoides monilifera* spp. *rotundata*) had invaded patches of the site. The soil substrate was characterised by Holocene parallel sand dunes with very little organic matter below the leaf litter layer (ca. 2 cm).

Five bags were buried under each of five bitou bush plants, five acacia plants and in five patches of bare sand at 10 cm below the ground surface. For the bitou bush and acacia conditions, the resin bags were buried within 10 cm of visible plant roots. Bags were left in situ for 10 days.

6.2.3 Compound extraction and GC-MS identification

The five resin bags from each plant or bare patch were pooled to produce five replicates from each condition (bitou bush, acacia and bare sand). To obtain a soil extract,

the five resin bags for each replicate were placed in conical flasks, DCM (250 ml) was added, and the flasks sealed for 24hours at room temperature 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).

Equal concentrations (4.13 g/ ml; w/v of DCM) of each extract were prepared and 0.5 µl was injected into a Varian 3700 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 x 0.25 mm) (SGE Australia) in the split mode with helium as the carrier gas. The oven temperature program began at 60 ˚C for 1 min, was increased by 4 ˚C/ min until 290 ˚C, and held at 290 ˚C for 15 mins. The compounds were subsequently identified by comparison with mass spectra and Kovats retention indices published in the electronic NIST (2002) and Sci Finder Scholar libraries (2006) and in Adams (2001).

6.2.4 Seedling growth bioassay

To emulate field concentrations of each extract, we prepared samples in the range of weights adsorbed by one resin bag in one day, which was between 1-5mg/ day. Concentrations of 1, 3 and 5 mg/ Petri dish were therefore used. Each sample was dissolved in DCM (1 ml) and added to a glass Petri dishes (9cm diameter) fitted with Whatman No. 1 filter paper. The DCM was allowed to evaporate in a fume cupboard for 15 mins; distilled water (2 ml) was added (producing concentrations of 1, 3 and 5 mg extract/ 2ml water), followed by 20 equidistant *Isolepis nodosa* (Rott.) R. Br (sedge; Cyperaceae) seeds. Seeds were collected from at least five sites within the Wollongong region. Two controls were included: one with distilled water (2 ml) and one where DCM (1 ml) had evaporated and distilled water (2 ml) added. Four replicate Petri dishes were conducted for each sample

and control type. Petri dishes were sealed with Parafilm® and incubated in a diurnal (12 hr/12 hr) temperature (15/25 ˚C) and light regime. Percentage germination and seedling root and shoot length were measured after 23 days.

6.2.5 Statistical analysis

6.2.5.1 Comparison of the chemical composition of each extract

The total weight of each extract and the amount of each compound (relative peak areas in the chromatogram) in each extract (n=5) in each condition (n=3) were compared using one-way ANOVA's with condition (bitou bush, acacia and bare sand) as a fixed factor (SPSS Version 12.0). The Student-Neumann-Keuls (SNK) test was conducted to test differences among conditions.

6.2.5.2 Seedling growth bioassay

Comparison of the effect of the water and DCM controls on *I. nodosa* germination, root and shoot length were assessed using ANOVA (SPSS Version 12.0). The DCM control replicates were employed as the zero concentration samples for the proceeding analyses.

The effects of increasing concentrations (0, 1, 3, 5 mg/ 2ml water) of the acacia soil, bitou bush soil and bare sand extracts on the germination percentages of *I. nodosa* were assessed using probit analysis (SPSS Version 12.0). The effects of each extract on the root and shoot lengths of *I. nodosa* were compared using ANOVA with condition as a fixed factor and concentration as a covariate in the model (SPSS Version 12.0).

6.3 Results

6.3.1 Comparison of the chemical composition of each extract

The resin bags extracted similar weights of material from below acacia and bitou bush canopies, and significantly less from the bare sand condition ($F_{2, 12}$=16.26, P<0.001; Fig. 6.2)

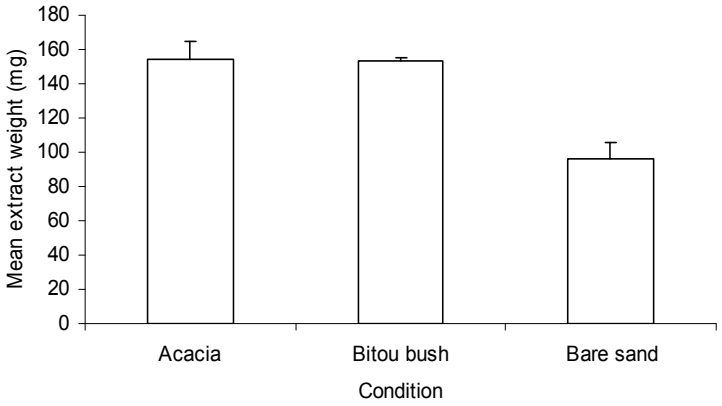
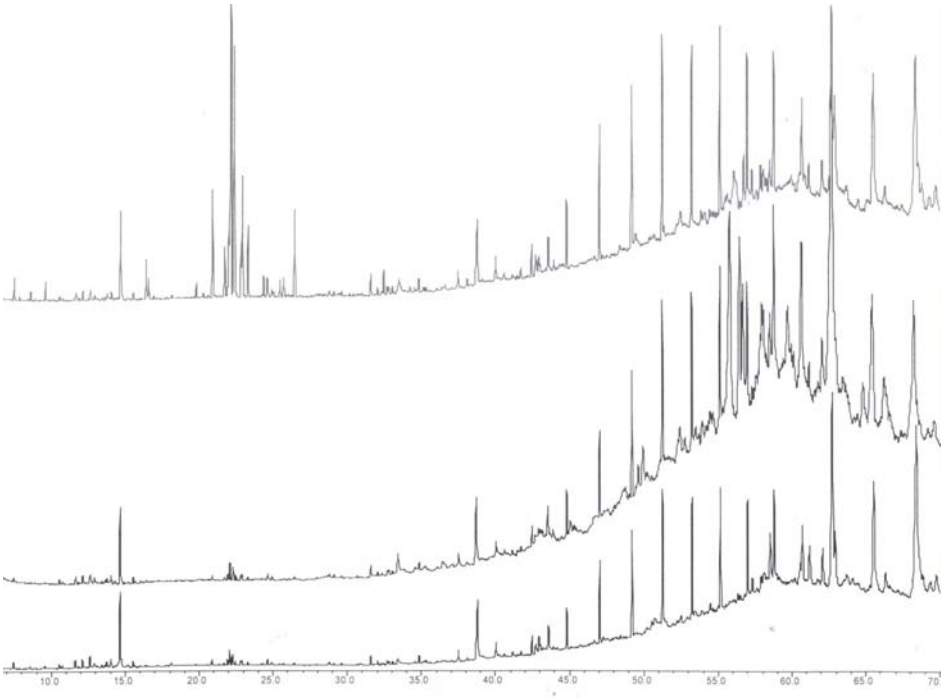


Figure 6.2: Mean weights of each extract from the acacia, bitou bush and bare sand conditions. Error bars represent one standard error.

Most of the hydrophobic compounds detected constituted similar proportions in the acacia, bitou bush and bare sand extracts (Fig. 6.3). Compounds common to all conditions included alkanes, alkanols, fatty acids and phytosterols. However significantly higher concentrations of terpenoids were found below bitou bush canopies, while higher concentrations of a phenolic compound was found below acacia canopies and more hexadecanoic and hexadecenoic acid was found in the bare sand compared to the other conditions studied (Table 6.1; Fig. 6.3).



Retention time (mins)

Figure 6.3: Representative gas chromatograms of the extracts from the resin bags placed in the bitou bush soil (top), acacia soil (middle) and bare sand (bottom).

Table 6.1: Mean percentage of, and ANOVA results comparing the proportional composition of each compound found to significantly differ between conditions.

Compound	Retention time (mins)	Mean (SEM) percentage of each compound in each condition: Acacia (A)	Bare sand (Ba)	Bitou bush (B)	*F* ratio (*df* =2,13)	*P* value	Post hoc tests
α-pinene	7.47	0.08	0.14	0.38	26.54	**<0.001**	A=Ba<B
camphene	7.83	0.01	0.01	0.05	16.26	**<0.001**	A=Ba<B
β pinene	8.57	0.02	0.02	0.10	15.08	**0.001**	A=Ba<B
3-carene	9.59	0.03	0.03	0.30	46.41	**<0.001**	A=Ba<B
a branched alkane	15.53	0.13	0.05	0.18	4.71	**0.031**	Ba≤ A≤ B
3-methoxy-*p*-cymene	16.39	0.05	0.08	0.38	12.99	**0.001**	A=Ba<B
2-methoxy-*p*-cymol	16.58	0.04	0.04	0.16	8.10	**0.006**	A=Ba<B
carvacrol ethyl ether	19.82	0.03	0.03	0.14	15.95	**<0.001**	A=Ba<B
7-*epi*-silphiperfol-5-ene	20.90	0.13	0.14	0.83	7.92	**0.006**	A=Ba<B
(+)-cycloisosativene	21.72	0.06	0.10	0.54	12.69	**0.001**	A=Ba<B
copaene	21.95	0.15	0.21	0.76	7.06	**0.009**	A=Ba<B
maaliene	22.10	0.35	0.47	3.08	9.06	**0.004**	A=Ba<B
α-isocomene	22.17	0.25	0.32	2.35	8.47	**0.005**	A=Ba<B
humulene	22.99	0.13	0.19	1.12	11.22	**0.002**	A=Ba<B
cymene	23.17	0.07	0.05	0.48	4.79	**0.030**	A=Ba<B
allo-aromadendrene	24.66	0.18	0.16	0.33	4.35	**0.038**	A=Ba<B
pentadecene	25.74	0.04	0.02	0.18	6.25	**0.014**	A=Ba<B
5-methoxycalamenene	32.48	0.10	0.08	0.11	4.29	**0.039**	A=Ba<B
5-hydroxycalamenene	36.20	0.04	0.03	0.05	4.15	**0.043**	Ba≤ A≤ B
hexadecanoic acid (Palmitic acid)	38.80	1.01	2.49	1.94	6.652	**0.011**	A≤ B≤ Ba
heptadecanoic acid (Margaric acid)	41.13	0.05	0.08	0.13	3.41	0.067	Ba<A<B
manool	42.72	0.19	0.36	0.50	7.47	**0.008**	A≤ Ba≤ B
9-hexadecenoic acid	42.91	0.18	0.50	0.43	4.81	**0.029**	A≤ B≤ Ba
a phenol	58.08	2.89	1.71	1.50	12.36	**0.001**	Ba=B<A

6.3.2 Seedling growth bioassay

There was no difference between the effect of the water and DCM controls on the germination percentage ($F_{1,6}$ =0.17; P=0.693), shoot length ($F_{1,6}$ =0.78; P=0.410) or root length ($F_{1,6}$ =0.16; P=0.707) of *I. nodosa.*

I. nodosa seed germination percentages significantly increased with acacia soil (Z=3.20; P<0.05) and bitou bush soil (Z=3.76; P<0.05) extract concentration and did not differ between the bare sand (Z= -0.85; P>0.05) extracts (Fig. 6.4). Although there was high variability in the germination success of *I. nodosa* and the Pearson's goodness of fit test showed that the probit models did not adequately represent the data, Fig 6.4 also

showed that there was an increase in the mean germination success with increasing concentrations of the acacia and bitou bush soil extracts.

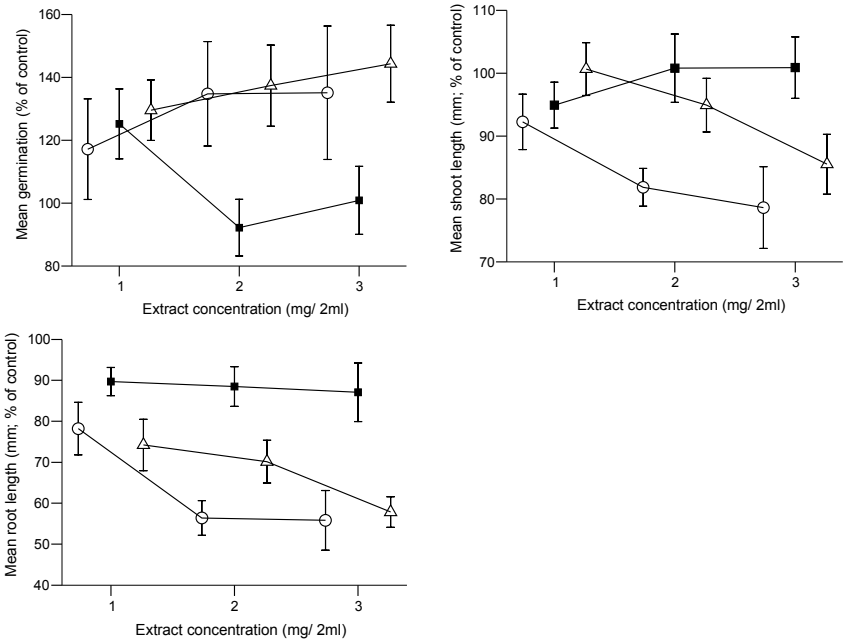


Figure 6.4: The germination percentages (a), shoot lengths (b) and root lengths (c) of *I. nodosa* (expressed as a percentage of the DCM control) with increasing concentrations of the bare sand (closed square), acacia soil (open circle) and bitou bush soil (open triangle) extracts.

Different concentrations of the different extracts had a significant effect on the shoot ($F_{2,50}$=3.97; P=0.025) and root ($F_{2,50}$=3.30; P=0.045) lengths of *I. nodosa.* There was no difference between the effect of each extract at 1mg/ 2ml (shoot length: $F_{2,12}$=0.32; P=0.731; root length: $F_{2,12}$=0.68; P=0.527; Figure 6.4. b and c). At 3mg/ 2ml, the acacia soil extract induced a significant reduction in shoot length ($F_{2,12}$=6.07 ; P=0.015; Fig. 6.4.b)

and both the acacia and bitou bush soil extracts induced significant inhibition of *I. nodosa* root length ($F_{2,12}$=4.40; P=0.037; Fig. 6.4.b) compared to the bare sand extracts. With extract concentrations of 5mg/ 2ml, both the acacia and bitou bush soil extracts continued to elicit an inhibitory response from *I. nodosa* root lengths ($F_{2,12}$=6.05; P=0.017; Fig. 6.4.c), and to a lesser extent by the shoot lengths ($F_{2,12}$=3.60; P=0.063; Fig. 6.4.b).

6.4 Discussion

This study demonstrated that different plants are likely to be associated with unique soil chemistry profiles which can function as mechanisms of interspecific interference competition. The hydrophobic chemical mixtures extracted from soil hosting an exotic invasive plant and a dominant indigenous species both inhibited the growth of an indigenous sedge, in contrast to the bare sand extract. This finding suggests that chemical interference competition may be widespread in sand dune vegetation, where resources are scarce, and may be an important influence on species dominance.

The exotic invasive shrub, bitou bush, was associated with a distinct hydrophobic soil chemical profile, particularly a higher concentration of sesquiterpenes compared to the indigenous acacia soil and bare sand extracts. Although these sesquiterpenes did not have a novel inhibitory effect on the indigenous sedge in this system, other studies have shown that the presence of these compounds in bitou bush invaded soil inhibit the growth of other sand dune species such as *Banksia integrifolia, Actities megalocarpa*, *Lomandra longifolia* and the acacia (*Acacia longifolia* var. *sophorae*) of the present study, more than hydrophobic extracts of the acacia dominated system (Chapter 4). Sesquiterpenes have been shown to have antimicrobial properties (Melcher *et al.* 2003; Scher *et al.* 2004) which may also confer a competitive advantage to bitou bush on the sand

dunes where mycorrhizal and bacterial symbioses are important for indigenous plant survival (Logan *et al.* 1989; Abe & Ishikawa 1999).

Not only does the addition of compounds by an exotic plant have the potential to affect the resident vegetation community, the absence of key compounds in invaded systems that are present of indigenous systems, may also drive community compositional shifts. In this study, the acacia soil extracts were distinguished by significantly higher amounts of an unknown phenolic compound compared to the bare sand and bitou bush soil extracts. The presence of phenolic compounds in the indigenous vegetated system may be integral to the indigenous community as they are known to affect litter decomposition (Hattenschwiler & Vitousek 2000), nutrient cycling (Hattenschwiler & Vitousek 2000), certain microbes (Inderjit & Dakshini 1991; Hattenschwiler & Vitousek 2000; Souto *et al.* 2000; Seneviratne & Jayasinghearachchi 2003), and act as allelopathic (Inderjit & Dakshini 1991; Leu *et al.* 2001; Chon *et al.* 2002) and anti-herbivore (Buchsbaum *et al.* 1984) agents.

Additionally, the chemical profile of the bare sand was characterised by high concentrations of hexadecanoic acid (palmitic acid) and 9-hexadecenoic acid, while the bitou bush invaded soil exhibited higher concentrations of heptadecanoic acid (margaric acid). Different abundances of fatty acids in soils suggests potential differences in, or effects on, the soil microbial community (Lucas Garcia *et al.* 2001; Karlinski *et al.* 2007), although current understanding of the physiological effects of different fatty acids is limited (Lucas Garcia *et al.* 2001). Palmitic acid is a common fatty acid in plants (Bolton *et al.* 1992; Liu & Huang 2004) and fungi (Ruess *et al.* 2005; Trepanier *et al.* 2005) which is transferred through the food chain to animals such as collembolan (Ruess *et al.* 2005). Bacteria such as *Aspergilli* spp. (Altieri *et al.* 2007) are inhibited by palmitic acid. The

presence of palmitic acid in bare sand patches suggests the absence or lack of small animal activity and possibly an altered microbial community compared to vegetated areas of the coastal sand dune systems studied. The effects of heptadecanoic (margaric) acid are less well known; however, similar species specific microbial effects and flow on effects through the food chain are postulated. Further investigation is required to elucidate the roles that these chemical compounds may directly and indirectly (via the microbial community) play in facilitating exotic plant invasion, indigenous community composition and ecosystem health.

Chapter 7: General discussion

The long and short term impacts of exotic plant invasion are hypothesized to alter resident plant community composition and loss of biodiversity (Costello *et al.* 2000). However there is a paucity of quantitative evidence on the longer term impacts , the possible evolution or adaptation of resident species (Carroll *et al.* 2005; Hoffmeister *et al.* 2005; Mealor & Hild 2006) and the underlying mechanisms (Levine *et al.* 2003) driving the observed changes. My research aimed to address some of these gaps by investigating the effect of South African bitou bush on the population dynamics and physiological health of several resident plant species and the potential for allelopathy and soil chemical interference as mechanisms of invasion. Understanding the ecology of invasions is imperative to implementation of successful management strategies. With regard to bitou bush, millions of dollars have been spent on the introduction of biological control agents, aerial herbicide spraying and on ground control efforts (DEC 2006). Although bitou bush has been controlled in some areas, approximately 80% of the New South Wales coast remains invaded and 72 plant species, populations and communities are threatened (DEC 2006).

7.1.1 Potential population, physiological and evolutionary impacts and mechanisms of plant invasion

The vulnerability of different life history stages of resident plant populations to an invader has gained minimal attention in the published literature, although some general hypotheses have emerged. Recruitment limitation has been suggested as a general impact of successful plant invaders (Yurkonis & Meiners 2004), and only a few studies have shown

that mature plant reproductive success can also be affected (Weiss 1984; Howard & Goldberg 2001). I therefore followed up on this research by examining the population size structures and the morphological and physiological characteristics of mature individuals of three taxonomically distinct resident plant species in invaded and non-invaded habitats. By investigating whether bitou bush had a morphological or physiological impact on resident plants, I was also incorporating studies of these characteristics as underlying micro-mechanisms of plant invasion. My findings concurred with Yurkonis and Meiners (2004) as there were significantly fewer smaller individuals in invaded habitats and the flower production, vegetative growth and physiological health of mature plants did not differ from non-invaded habitats, suggesting that bitou bush affected the establishment (seedling) or recruitment (germination) success of resident plant species (Chapter 2). Bitou bush forms monocultures on the New South Wales coast if left unmanaged and recruitment limitation is proposed as the population level mechanism driving this outcome. By exploring the variability in reproductive output and vegetative growth of resident mature species we also detected the possibility for natural selection for more tolerant individuals in invaded habitats. The flower abundance of *C. alba* was significantly more variable within invaded habitats compared to within non-invaded habitats, suggesting that more susceptible genotypes which reproduce less may drop out of the system, leaving more tolerant individuals that are able to reproduce as in the non-invaded habitat. Further molecular analysis is required to determine whether this species may be adapting to the new environmental conditions induced by the invasion of bitou bush.

Ecosystem property changes such as nitrification (Vitousek & Walker 1989; Evans *et al.* 2001; Ehrenfeld 2003; Standish *et al.* 2004; Yelenik *et al.* 2004; Lindsay & French 2005), altered decomposition rates (Lindsay & French 2004) and microclimate (Lindsay &

French 2004) and leaf litter depth (Minchinton *et al.* 2006) are often cited as impacts and mechanisms of exotic plant invasion. In attempt to link pattern to process, I further explored whether bitou bush invasion altered ecosystem properties associated with three indigenous plant species and whether there were correlating changes in the photosynthetic capacities of the plants, measured in-situ and after dark adaption. I found that for each indigenous species (five per site), over ten different sites, five invaded and five non-invaded, there was no consistent difference in the leaf litter depth, soil pH, nitrates, ammonium or phosphorus levels between invaded and non-invaded habitats (Chapter 3). Significant differences in all of these parameters were found between sites. However in fore dune invaded sites, there was a significant increase in canopy cover above *Correa alba*, and in hind dune invaded sites there was a significant decrease in canopy cover above *Monotoca elliptica* (Chapter 3). No difference in the canopy cover above *Lomandra longifolia* (a rush of both fore dune and hind dune distribution) was found between habitats. I suspect that the changes to the canopy cover were a function of the plant height and habitat: *C. alba* is a canopy shrub of the foredune and *M. elliptica* is an understorey, small tree. Bitou bush is therefore likely to overgrow canopy species of the foredune and understorey shrubs of the hind dune. Swamping has been suggested as an interference mechanism of invasion by several other authors (Williamson 1996; Siemann & Rogers 2003; Reinhart *et al.* 2006; Coleman & Levine 2007) however the effect on resident plant photosynthetic capacity or other micro-mechanisms such as relative growth rate or leaf area (Shainsky & Radosevich 2003) are rarely incorporated into such studies. Moreover, the physiological or morphological effect of increased irradiance of mature, tall, understorey species that may have resulted in the loss, or lack of regeneration of canopy species as a result of exotic plant invasion has not been studied to my knowledge.

Short term fluctuations in plant photosynthetic parameters have been documented as occurring in response to changing light environments (Pearcy & Sims 1994; Murchie & Horton 1997; Watling *et al.* 1997; Rozendaal *et al.* 2006). Additionally, longer term shifts from shade to sun plant photosynthesis dynamics, and vice versa, may also occur in response to sustained changes in irradiance (Boardman 1977). Based on my findings that bitou bush invasion alters the canopy cover and hence light environment differentially for different species, I expected differential shifts in either quantum efficiency or Fv/Fm (long term changes) or Pmax (short term change) in plants studied: from sun to shade characteristics in *C. alba* and shade to sun characteristics in *M. elliptica*. However I detected no consistent short or long term changes in the mean photosynthetic capacities of any of the three resident species in bitou bush invaded habitats (Chapter 3). I did however find that there was less variability in photosynthetic capacity of *C. alba* plants within the invaded habitat compared to within the non-invaded habitat. Further assessment of the ground incident light and temperature below the indigenous plant canopies and bitou bush canopies on the fore dune showed that the microclimate below bitou bush canopies was more homogeneous and moderate than below indigenous canopies, which may explain the more homogeneous photosynthetic capacities of *C. alba* plants of invaded fore dunes.

Assessment of the differences in the variability of traits, as well as differences between the trait means between habitats (or environmental stresses) provides insight into acclimation of species (Bazzaz 1996; Stanton *et al.* 2000), which alludes to the genetic micro-mechanisms underlying successful invasion. Our study of the seasonal photosynthetic capacities of *C. alba, M. elliptica* and *L. longifolia* in invaded and non-invaded habitats revealed that the mean Fv/Fm of these species did not differ between habitats, however there was less variability in Fv/Fm for all species in invaded habitats

particularly in August. Reduced variability or homogeneity of traits has been suggested as a potential threat to the tolerance of future environmental stress (Hoffman & Parsons 1989; Kozlowski & Pallardy 2002). Coastal plants such as studied here have evolved to tolerate the stressful coastal environment (Ecke & Rydin 2000) and demonstrate high phenotypic plasticity (Ernst 1985; Gray 1985) which confers future tolerance. If the maternal environment of future generations of indigenous species is moderated to be less stressful, the stress tolerance of future generations may be lost (Roach & Wulff 1987; Weiner *et al.* 1997; Moriuchi & Winn 2005). Maintenance of stress tolerance in these species is even more important considering the likelihood of future environmental change (Hughes 2003).

Therefore, my investigations into the impacts of plant invasion have quantified the short and longer term impacts on plant population sustainability, but also lend insight into the possible evolutionary effects of bitou bush on resident species. Further genetic assessment is required to elucidate the possible evolutionary impacts of plant invasion alluded to in these studies.

7.1.2 Soil chemical interference and allelopathy as mechanisms of invasion

Mechanisms driving the invasion of exotic plants have been traditionally cited as enemy release (Darwin 1859; Keane & Crawley 2002; Hierro & Callaway 2003; Liu & Stiling 2006), the evolution of increased competitive ability (Blossey & Notzold 1995; Siemann & Rogers 2001; Thebaud & Simberloff 2001) or superior resource acquisition and competition (Amarasekare 2002). Interference and indirect competition are often ignored as potential influences on interspecific interactions and community composition (Amarasekare 2002; Hierro & Callaway 2003; Inderjit & Callaway 2003; Meiners 2007) despite the ubiquity in nature (Amarasekare 2002). Although allelopathy is probably the most studied

form of interference competition between plants, incorporation in to plant invasion theory has been slow and primarily based on difficulties associated with unequivocal detection (Reigosa *et al.* 1996). Critiques of the "grind and find" (Romeo 2000) and bioassay (Inderjit & Weston 2000; Inderjit & Nilsen 2003) methodologies have surfaced based on the lack of appropriate controls (Williamson & Richardson 1988) and potential modification of plant derived compounds (Blum *et al.* 1993; Huang *et al.* 1999; Inderjit 2005) . For suspected soil localized allelochemicals, incorporation of the soil substrate into experiments has been suggested (Pellissier 1998; Inderjit 2001; Kobayashi 2004) and even a semantic shift to soil chemical ecology proposed (Inderjit & Weiner 2001).

Preliminary quantitative evidence suggests that bitou bush leaf litter and plant extracts inhibit plant growth (Copeland 1984; Hughes 1998; Vranjic *et al.* 2000), however the findings of these studies were marred by small sample sizes, ambiguous results and rudimentary methodology. Similarly, allelopathy has been implicated in anecdotal observations of bitou bush monoculture formation and failure of indigenous plants to establish following bitou bush control. Hence, I conducted a carefully designed series of experiments in attempt to clarify whether bitou bush is allelopathic towards Australian resident species and to try and identify potential allelochemicals in-situ. Key elements of the studies were comparisons of chemical profiles and bioactivity of extracts from bitou bush and the dominant indigenous species of the invaded system, coastal acacia (*Acacia longifolia* var. *sophorae*), testing of extracts on five species indigenous to the invaded system, and assessment of the compounds present in both plant and the associated soils. There are few documented studies of allelopathy which adopt this ecosystem based approach that allows for inferences on the role of allelopathy in shaping community structure and exotic plant dominance.

Bioassays of the hydrophobic to hydrophilic extracts of bitou bush and acacia roots, leaves and soil revealed that both plants contain phytotoxic mixtures of compounds that inhibited the growth of all six test species (Chapter 4). More importantly from an ecological perspective, extracts from both the root and soil of bitou bush inhibited the growth of four of the indigenous test species. Comparable extracts from the acacia also inhibited one these species, *I. nodosa* (Chapter 4). We propose that inhibition by comparable solvent extracts from the root and soil of one species is suggestive of allelopathy. Similarly, both the bitou bush and acacia soil extracts inhibited the growth of two test species: *B. integrifolia* seedling growth was affected by both acacia and bitou bush; *L. longifolia* was inhibited by the acacia soil extract; and *A. longifolia* var. *sophorae* (acacia) seedling growth was inhibited by the bitou bush soil (Chapter 4). Therefore, although it appears that chemical interference between plants can occur irrespective of plant origin (against *I. nodosa* in this case), bitou bush was allelopathic to a greater number of indigenous species and had an indirect affect against the dominant acacia, which is may translate to the displacement of indigenous species in the field.

As the hydrophobic extracts of the acacia and bitou bush roots and soils were most inhibitory, I further explored the chemical composition of these components. GC-MS analyses of the dichloromethane (DCM) extracts of the roots and soils of the acacia revealed that three compounds were exclusive to the bitou bush root and soil, and seven compounds were common to the bitou bush and acacia roots but only present in the bitou bush soil (Chapter 5). The compounds unique to the bitou bush invaded soil were all members of the sesqui- or diterpenoid family. Several of these compounds were found to inhibit the seedling growth of *Isolepis nodosa*. Of particular interest were 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 and also exhibited phytotoxic activity (Chapter 5).

To further confirm the compounds identified in the bitou bush invaded system via the solvent extraction technique, I devised a novel method to trap soil hydrophobic compounds in-situ. This technique utilised bags filled with hydrophobic adsorbent resin to assess the chemical profile associated with bitou bush invaded, indigenous vegetated soils and non-vegetated soils. GC-MS analyses of the adsorbed compounds, as removed by DCM, showed that the bitou bush invaded soils contained significantly higher amounts of sesquiterpenes and the indigenous vegetated soils contained significantly higher amounts of certain phenols than the unvegetated soils, which had higher concentrations of hexadecanoic acid (Chapter 6). Bioassays of the DCM extracted mixture of compounds, at concentrations approximating the range of weights collected by one resin bag on one day (1, 3, 5mg), elicited a negative response by *I. nodosa* to both the indigenous vegetated soil extract and bitou bush invaded soil extracts, compared to the non-vegetated soil extract which had the same effect on *I. nodosa* growth with increasing concentrations as found for the water control (Chapter 6). Hence, as found for the solvent extracts, *I. nodosa* was susceptible to the soil of both invaded and non-invaded conditions demonstrating the presence of chemical interference competition in both natural and invaded systems.

7.1.3 Conclusions

Collation of the aforementioned studies, suggests that although chemical interference between species is likely to guide community composition, the invasion of exotic plants may introduce a new suite, or in some cases higher concentration of allelochemicals, and perhaps preclude the input of other compounds characteristic of the

indigenous system. This alteration of the soil chemical profile, or habitat construction, has been shown here to affect the establishment and growth of indigenous species which is likely to drive the exotic plant invasion and potentially facilitate monoculture formation. The population size structure analyses and assessment of mature plant health suggested that the recruitment stage is more likely to be susceptible to the invasion of bitou bush, and we suggest that soil chemical interference or allelopathy is likely to one mechanism driving this impact.

7.1.4 Management implications

In light of these findings, and to promote the restoration of pre-bitou bush invaded coastal ecosystems in New South Wales, I suggest that a lag time or burning follow the removal of bitou bush to volatilise the terpenes which are likely to inhibit indigenous plant establishment. Planting of juvenile indigenous species is also suggested to avoid the periods of vulnerability – the germination and seedling growth stages, as detected in this study.

7.1.5 Future directions

The altered variability in the physiological and morphological responses of indigenous plant species to bitou bush invasion could have evolutionary implications for these species if bitou bush is not managed on the eastern Australian coast. For example reduced flower production of some individuals of *C. alba* in bitou bush invaded habitats could result in the loss of susceptible genotypes and therefore potentially loss of genetic diversity in this species which could have further species survival consequences. The reduced variability in photosynthetic stress may also have genetic implications if bitou bush is not managed as dune species, such as those of this study, may lose the ability to tolerate

bouts of environmental stress which are particularly likely to occur in light of future global climate change. Population genetic studies of indigenous plants are therefore suggested to determine whether the invasion of bitou bush has, or may have, genetic effects on resident plants of the new host environment.

My studies into the potential allelopathic effects of bitou bush invasion suggest that bitou bush may be allelopathic in the new host environment, however further studies could be conducted to elucidate the mode of release, soil persistence, seed or seedling uptake and the biochemical or morphological effects of the putative hydrophobic allelochemicals. Additionally, the presence of hydrophilic allelochemicals in bitou bush invaded systems could be investigated. Our solvent extract bioassays did suggest potential allelopathy by the hydrophilic extracts of bitou bush roots and soil; of particular interest was the indirect soil chemical interference on some species, suggested by the inhibition of the soil extracts alone. To complement the laboratory based studies on allelopathy, field or pot trials are also suggested to further incorporate other possible abiotic and biotic factors that could mitigate the potential allelopathic effects described here and show accumulation of allelochemicals after the introduction of bitou bush.

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