Do mutualists matter? The role of pollinators, seed dispersers and belowground symbionts in the invasion success of *Acacia*

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Abstract

Plant species introduced to new locations may lose their natural enemies but can also leave behind important mutualists. Here, I take a novel comparative approach to identify the potential role of mutualistic interactions in determining invasion outcomes. I examine the strength of pollination, seed dispersal and belowground symbioses with nitrogen-fixing bacteria (rhizobia) across three species that vary in invasion success in both their introduced and native range. I used species of Australian *Acacia* introduced to New Zealand. I hypothesised that if interactions with mutualists are important for plant invasion then species would vary in the strength of interactions with one or more of the groups of mutualists I examined, and that the pattern of variation would correlate with the degree to which they have established and spread in New Zealand. At each stage I also consider the potentially mediating influence of natural enemies.

For *A. dealbata*, a highly invasive species, and *A. baileyana*, a species that is widely naturalised in New Zealand, I found no differences in any of the variables I examined in relation to pollination and predispersal seed predation. However, for *A. pravissima*, currently considered a casual species in New Zealand, pre-dispersal seed predation was lower in New Zealand and overall seed production was much higher relative to conspecifics in Australia, and relative to the other two species. In relation to seed dispersal I found that the three species, which are all adapted for dispersal by ants (myrmecochory), were able to form dispersal mutualisms in New Zealand, potentially to the same degree as in Australia. Seed predation following seed fall was also lower for species in New Zealand than in Australia. There was no variation between the three species in seed removal associated with either dispersal or predation. By examining species' growth and nodulation with rhizobia in both Australia and New Zealand I found that their ability to spread away from introduction sites could be limited by the availability of rhizobia in New Zealand, relative to Australia. However, there were again no differences between species.

This is the first study to have directly measured mutualistic interactions across species that vary in invasive success in both their native and introduced range. I demonstrated that species introduced to new locations are able to establish mutualistic interactions with pollinators and dispersers to the same degree as in their native range. I also found the first direct evidence that the availability of rhizobia could limit species' abilities to colonise new sites in the introduced range. However, mutualistic associations could not explain the variable invasive success of each species. Overall, these findings suggest that mutualistic interactions may be important for alien plant establishment, but alone cannot explain invasion outcomes. Instead, it is likely that invasive success is determined by a combination of biotic, abiotic and human factors, with the ability to establish mutualistic interactions just one component necessary for successful establishment and spread. These findings underline the importance of such broad geographical and comparative studies in attempts to elucidate drivers of invasion.

Keywords: *Acacia*, alien, Australia, antagonisms, belowground symbioses, biogeographic, comparative, establishment, exotic, invasion, mutualisms, myrmecochory, natural enemies, naturalisation, New Zealand, pollination, predation, rhizobia, seed dispersal, spread.

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Chapter 1 - Introduction

1.1 From introduction to invasion

Plant species are frequently introduced to areas outside their native range for use in forestry, land rehabilitation or the ornamental trade. Although many plant species only persist in new areas under cultivation, a small subset establishes self-sustaining populations and become naturalised and a still smaller subset spread away from the area of introduction to become invasive (Williamson & Fitter 1996; Richardson *et al.* 2000b; Theoharides & Dukes 2007). Where invasive, alien plant species can have substantial impacts on natural ecosystems by changing community composition, altering water and nutrient cycles and disrupting fire regimes (Levine *et al.* 2003; Vilà *et al.* 2011; Pyšek *et al.* 2012). Understanding the mechanisms that enable species to establish in new regions and spread away from the site of introduction is therefore a fundamental goal of invasion biology (Rejmánek & Richardson 1996; Rejmánek 1999; Richardson & Pyšek 2012).

In order to successfully establish and spread a plant species must be able to reproduce, disperse to new locations and establish and survive in those new locations (Figure 1.1; Richardson *et al.* 2000a; Blackburn *et al.* 2011; Richardson & Pyšek 2012). For this reason, traits associated with successful reproduction and dispersal are often considered crucial for invasion (Rejmánek 1996; Lloret *et al.* 2005; Pyšek & Richardson 2007; Dawson *et al.* 2009; Pyšek *et al.* 2009a). In particular, the ability to reproduce and disperse seeds without the requirement for animal mutualists is expected to confer an advantage on alien plant species, since it reduces the need to establish new associations with a potentially different fauna. For example, autonomous self-fertilisation and the ability to reproduce vegetatively has been identified as contributing to successful reproduction and thus establishment into new locations for alien plant species in the USA (Reichard & Hamilton 1997; Burns 2006; van Kleunen & Johnson 2007), while adaptation for wind dispersal is implicated in successful spread in Mediterranean ecosystems (Lloret *et al.* 2005). However, species reliant on biotic interactions for successful establishment and spread have also become invasive where introduced.

Previously, research aimed at understanding the potential for biotic interactions to influence plant invasion has focussed on negative interactions with natural enemies. In particular, the enemy release hypothesis has received considerable attention (Elton 1958;

Keane & Crawley 2002). This posits that species introduced to new locations leave behind natural enemies that regulate populations in their native range. For example, those that can influence a species' ability to establish and spread (Figure 1.1) include herbivores (Rees & Paynter 1997; Agrawal *et al.* 2005; Stastny *et al.* 2005), including seed predators (Fenner & Lee 2001; Wolfe 2002), and soil pathogens (Klironomos 2002; Agrawal *et al.* 2005; Callaway *et al.* 2011). However, there is increasing recognition that interactions with natural enemies alone are insufficient to explain the variable success of introduced plant species, as all species should be equally free from natural enemies in the introduced range yet not all species invade (Hierro *et al.* 2005; Parker & Gilbert 2007). Furthermore, if species escape their natural enemies they may also leave behind important mutualistic partners (Richardson *et al.* 2000a; Mitchell *et al.* 2006). Increasing attention has therefore focussed on the potential role that loss of positive interactions with mutualists might play in alien plant establishment and spread (Simberloff & Von Holle 1999; Richardson *et al.* 2000a; Parker & Haubensak 2002; Morris *et al.* 2007; Dickie *et al.* 2010; Abe *et al.* 2011; Callaway *et al.* 2011).

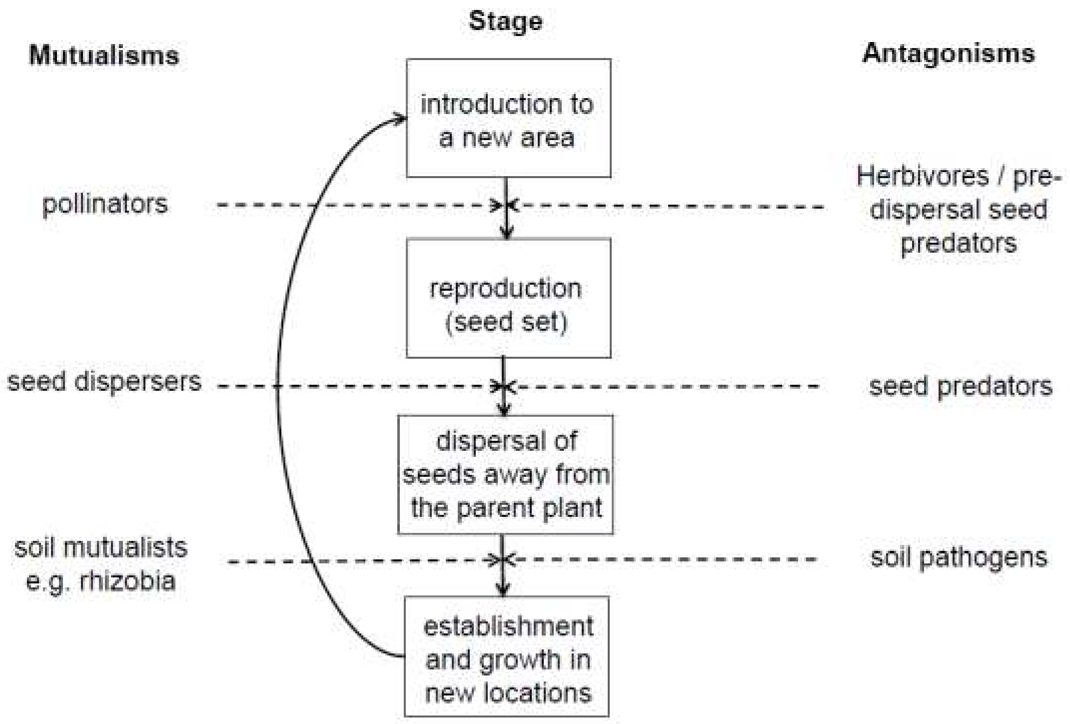

Figure 1.1 Four key stages from introduction to invasion (boxes). The solid vertical arrows represent the three transition phases focussed on in this thesis. The dashed arrows highlight biotic interactions important at each stage.

1.2 The role of mutualisms

Mutualistic interactions that can facilitate successful establishment and spread include those a plant has with pollinators, seed dispersers and belowground symbionts (Figure 1.1). Species dependent on one or more mutualisms introduced to areas without any suitable mutualists are expected to fail to establish and spread, and there is evidence that the loss of mutualistic partners may outweigh the advantages of leaving behind natural enemies (Morris *et al.* 2007).

1.2.1 Pollination

For species reliant on biotic pollination for reproduction, their ability to form effective pollination mutualisms could be an important barrier to establishment when introduced to new regions. For example, lack of pollinators is implicated in the failure of clovers to establish initially into New Zealand (Hopkins 1914; Gardner & Early 1996). Pollination success can also be an important determinant of species' spread in those new regions, since it influences the number and fitness of propagules available for dispersal.

The success of pollination depends on both the quantity and quality of pollen available (Aizen & Harder 2007). The quantity of pollen deposited may decrease in new locations if aspects of species' floral morphology make them inaccessible or unattractive to new pollinator assemblages (Bjerknes *et al.* 2007; Küster *et al.* 2008), or because the initially low densities that commonly characterise early stages of invasion also fail to attract foraging species (Mustajärvi *et al.* 2001; Firestone & Jasieniuk 2012). The quality of pollen deposited may decrease due to differences in the availability and genetic variability of conspecific pollen donors (Parker 1997; Broadhurst *et al.* 2008; Firestone & Jasieniuk 2012), the numbers of congeners or other species that share the same pollinators within close proximity to the plant (Brown & Mitchell 2001), and the foraging behaviour of the pollinator involved (Grant *et al.* 1994; Burns *et al.* 2011). For these reasons, a generalised pollination syndrome and self-compatible breeding system are expected to promote invasion success (Baker 1974; van Kleunen & Johnson 2007; Burns *et al.* 2011; Gibson *et al.* 2011), since they increase the likelihood species will attract pollinators in new locations and decrease reliance on the effectiveness of those new associations.

However, although there are rare examples of species failing to initially establish, most species are expected to readily form mutualistic interactions with pollinators (Baker 1974;

Stout *et al.* 2006; Abe *et al.* 2011). This is largely because generalisation is common in pollination syndromes (Waser *et al.* 1996; Vázquez *et al.* 2009) and many generalist pollinators have been widely introduced (Goulson 2003). Nevertheless, despite the advantages of self-compatibility and evidence that it predominates in alien flora (van Kleunen & Johnson 2007; Burns *et al.* 2011; Hao *et al.* 2011; Petanidou *et al.* 2012), some important invaders are highly outcrossing. Therefore, the importance of pollination mutualisms in determining invasion outcomes is still unclear.

1.2.2 Seed dispersal

The ability to spread away from introduction sites and colonise new locations within the introduced range is an important stage in the transition from introduction to invasion (Figure 1.1; Richardson *et al.* 2000b; Richardson & Pyšek 2012). For species with a zoochoric dispersal syndrome the potential for animal-mediated dispersal may therefore pose an important barrier to invasion. For example, the recent invasion of Persian walnut (*Juglans regia* L.) in Europe, despite a long history of planting, is attributed to an increase in numbers of an avian seed disperser (Lenda *et al.* 2012). Dispersal not only facilitates the colonisation of new locations but can also decrease the risk of seed predation (Janzen 1970; Giladi 2006) and improve the micro-site conditions available for germination (Wenny 2001; Giladi 2006; Berg-Binder & Suarez 2012).

As with pollination, a number of factors can influence the probability a seed is dispersed and the combination of species' traits and disperser assemblage could be an important determinant of invasion outcomes. Seed size (Buckley *et al.* 2003), the timing of seed release (Andersen & Ashton 1985; Hughes & Westoby 1990) and the density of seeds available (Hulme 1997) have been identified as important, though generalisations have been difficult to find. For example, small seed size was positively correlated with pine (*Pinus* sp.) invasion success in one study (Rejmánek & Richardson 1996), but other studies found no relationship between seed size and invasion outcomes in other systems (Hughes & Westoby 1992a; Buckley *et al.* 2003). It is likely that attributes that confer invasion success are highly context specific and vary according to both the plant species involved and the landscape they are introduced into. The factors controlling success may also vary depending on the dispersal syndrome, since a small seed size may increase dispersal distance in wind dispersed species, but decrease the probability of discovery in species reliant on animals for dispersal.

Within species that show a zoochoric dispersal syndrome, vertebrate dispersal is identified as important (Rejmánek 1996; Renne *et al.* 2002) because it may be more likely to facilitate long-distance spread (Dawson *et al.* 2009). However, numerous invasive species are myrmecochorous and rely on ants for both the dispersal of seeds away from parent plants and for the burial of seeds and so incorporation into the seedbank. While there is evidence that introduced myrmecochores are able to form dispersal mutualisms in new locations (Holmes 1990; Jensen & Six 2006), the extent to which their ability to do so might promote or hinder plant invasion remains unknown.

1.2.3 Belowground symbioses

The suitability of a habitat for species dispersed to new locations can be strongly influenced by the soil biota present. Leguminous plant species may be particularly susceptible to establishment failure in new locations due to their reliance on forming mutualistic associations with nitrogen-fixing bacteria in the soil (rhizobia), which is highlighted by the use of rhizobial inoculants to promote plant growth in agriculture and forestry (Umali-Garcia *et al.* 1988; Turk *et al.* 1993; Tahir *et al.* 2009). Therefore, although most work has focussed on the role of losing soil pathogens in determining the invasive success of some species (Klironomos 2002; Reinhart *et al.* 2003; Reinhart & Callaway 2006), the role of rhizobia in facilitating plant establishment into new ranges has received increasing attention (Parker 2001; Rodríguez-Echeverría *et al.* 2009; Birnbaum *et al.* 2012).

Rhizobia are free-living in the soil and the symbiosis between rhizobia and their plant hosts occurs when the bacteria infect the roots of plants, forming structures called nodules in which nitrogen fixation occurs. The plant profits from increased nitrogen uptake and subsequent fitness improvements (Burdon *et al.* 1999), while the bacteria are hypothesised to benefit from the provision of other nutrients and protection (van Rhijn & Vanderleyden 1995). Successful invaders are expected to be able to nodulate with a wide range of strains and at lower rhizobial densities (Rodríguez-Echeverría *et al.* 2011), i.e. show high symbiotic promiscuity. They should also exhibit lower rates of antagonistic relationships (Klironomos 2002), such as those with essentially parasitic rhizobia (Thrall *et al.* 2007) and soil pathogens.

Currently, our understanding of the extent to which species may establish effective mutualisms in new locations is limited, since most previous work has focussed on a few highly invasive species (Parker *et al.* 2007; Rodríguez-Echeverría *et al.* 2011; Birnbaum *et al.* 2012), or on species' response to inoculation under controlled conditions in agricultural research (Turk *et al.* 1993; Tahir *et al.* 2009; Boukhatem *et al.* 2012). In addition, studies that specifically target the role of rhizobia in plant invasion often focus on mutualistic interactions formed by established populations (Rodríguez-Echeverría 2010; Birnbaum *et al.* 2012), which could mask the potential importance of soil biota for invasion in two key ways. First, such populations represent the successes rather than the failures, and so individuals that have encountered suitable mutualists. The frequency with which species encounter suitable mutualists could be a determinant of invasive success. Second, for species growing in cultivation their initial establishment was likely buffered by human intervention, which could give plants the opportunity to build up necessary symbionts in the soil over time. Therefore, the extent to which soil biota might limit species spread away from sites of introduction may be a more relevant test of their role in plant invasion.

1.3 Defining the unit of comparison

Most research aimed at understanding processes driving invasion success has focused on species in their introduced range. However, neither the positive impacts of escape from natural enemies nor the negative impacts of losing mutualists can be demonstrated without some measure and understanding of the ecological significance of those interactions on species population dynamics, which requires examining them under natural conditions in their native ranges (Hierro *et al.* 2005). Although the importance of such comparative studies is increasingly recognised, there are still relatively few field studies that examine species interactions in both their native and introduced range (though see Birnbaum *et al.* 2012; Petanidou *et al.* 2012). Therefore, an important gap in current research is the extent to which the strength of biotic interactions varies for alien species in their introduced relative to their native range.

In addition to a paucity of studies that examine species in their native and introduced range, there are also few that examine species that have shown varying invasion outcomes. Currently, most research is focused on the few species that are widespread and abundant invaders where introduced and therefore have the greatest impact (Richardson & Pyšek 2012), but understanding why some species are successful in new locations requires an understanding of differences between those species and ones that have shown more limited invasion success (Burns 2006; Richardson & Pyšek 2012). Although there are several reviews and meta-analyses that examine differences between invasive and non-invasive species (Pyšek & Richardson 2007; van Kleunen *et al.* 2010; Gibson *et al.* 2011), field studies that explicitly test these differences are again limited. Of those that do, the focus is on comparing invasive species in their invaded range with their native congeners (Vanparys *et al.* 2008; Powell *et al.* 2011; Vervoort *et al.* 2011) or on invasive species only (Ward *et al.* 2012). Comparing differences between congeners is important, since it can help control for variation in life-history traits and evolutionary histories (Agrawal & Kotanen 2003; Burns 2006; Muth & Pigliucci 2006; Powell *et al.* 2011). However, focussing on only invasive species makes it difficult to understand why some species fail to invade where others succeed. In addition, native congeners may themselves be invasive somewhere else in the world, meaning that comparing invasive species with native congeners in the invaded range runs the risk of comparing invasive species with other invasive species (Muth & Pigliucci 2006). A more pertinent comparison may therefore be between invasive alien species and non-invasive alien congeners (Theoharides & Dukes 2007).

In this thesis, I aim to undertake a comparative approach to understanding the importance of mutualistic interactions for plant invasion success. I will focus on the three transition phases outlined in Figure 1.1 and compare species exhibiting varying levels of success in their introduced range with the same species in their native range to determine whether differences in the strength of mutualistic interactions in the introduced versus the native range can explain the variable invasion outcomes shown by alien plant species. In addition, although mutualistic interactions are the focus of the thesis, I also take the potential importance of natural enemies into account, since they may disproportionally influence species' success at any of the stages I examine and mask any variation between species in their mutualistic associations. I examine species of Australian *Acacia* introduced to New Zealand.

1.4 The Australian *Acacia* as a model system

Acacia is the second largest genus within the Leguminosae comprising in excess of 1300 species worldwide with a natural distribution that spans Africa, Australia, Asia and the Americas (Maslin *et al.* 2003). It is a polyphyletic group that was first described in the

18th Century (Miller 1754) but has since undergone several taxonomic revisions. The classification of this genus is still the subject of some debate but in this thesis I use the term *Acacia* to refer to the 1012 species of Australian *Acacia* in the subgenus Phyllodineae, following Richardson *et al.* (2011). Around 95% of these species are endemic to Australia (Maslin 2001) and, within Australia, species show a wide distribution that spans all major habitat types and biomes, ranging from cool coastal or mountainous climates to the hot, dry shrublands.

Acacia have a variety of uses and as a result have been widely introduced outside their native range. Where introduced, species are of considerable commercial value within the ornamental trade, the tannin industry and for timber production (de Wit *et al.* 2001; Griffin *et al.* 2011; Kull *et al.* 2011). They have also been planted for soil stabilisation and habitat restoration (Rodríguez-Echeverría *et al.* 2009) and are widely used by rural communities for firewood, construction and animal fodder (Kull *et al.* 2011). An estimated 386 species have been recorded outside of Australia, 43 of which are currently considered naturalised and 23 of which are recognised as invasive and the *Acacia* are well represented in the global invasive flora (Richardson & Rejmánek 2011). Where invasive, species threaten native biodiversity and important ecosystem services, such as nutrient cycling, water balance and the provision of timber and food (de Wit *et al.* 2001; Richardson & Van Wilgen 2004; Marchante *et al.* 2009; Le Maitre *et al.* 2011). Consequently, there is increasing attention on identifying what determines the invasive success of species within this group (Richardson *et al.* 2011).

1.4.1 *Acacia* in New Zealand

Despite their geographic proximity, Australia and New Zealand are biologically very different. While Australia supports a diverse fauna, the fauna of New Zealand is relatively depauperate, which has been exacerbated by its history of human settlement (Clout & Hay 1989). Of particular relevance to this thesis is the limited suite of native pollinators and dispersers present, relative to Australia (Webb & Kelly 1993). In addition, although the fossil pollen record indicates *Acacia* were present in New Zealand until sometime during the Pleistocene (Mildenhall 1972; Lee *et al.* 2001), there are currently no extant native species of *Acacia* in New Zealand, meaning that there is unlikely to be an existing suite of mutualists pre-adapted to introduced *Acacia*. Despite this, numerous Australian plant species have become successful invaders in New Zealand, including some species of *Acacia*.

As a result of European colonisation many species of *Acacia* were introduced to New Zealand during the 1800s for forestry trials, agricultural windbreaks, as nurse trees for other tree species, soil stabilisation, use in the tanning process and for ornamental purposes (Ludlam 1865; Papers Past New Zealand 2012). Since then, at least 150 species of Australian *Acacia* have been introduced (Appendix A; Diez *et al.* 2009). Of those species, 17 have naturalised (Table 1.1), defined as having established self-sustaining populations (Howell & Sawyer 2006) and eight are sufficiently widespread to be classed as environmental weeds (Howell 2008). All naturalised species are native to the more temperate region of south eastern Australia (Table 1.1).

1.4.2 Defining species' status

Definitions of alien species' status can vary between studies and cause confusion in the interpretation of results. Consequently, a number of conceptual frameworks for defining alien species' status have been proposed (e.g. Richardson *et al.* 2000b; Colautti & MacIsaac 2004; Blackburn *et al.* 2011). One commonly used classification is that given in Richardson *et al.* (2000b), which views the process of invasion as a series of barriers a species must overcome between introduction and invasion and focuses on the extent to which species have spread away from introduction sites:

Introduction	A plant has been transported by humans to new locations.
Naturalisation	Various biotic and abiotic barriers to establishment have been overcome. Species are able to reproduce freely and have established self-sustaining populations in the vicinity of the introduced plant.
Invasion	Species have spread away from the site of introduction (approximate scales: > 100 m over < 50 years for taxa spreading by seeds and other propagules) and established self-sustaining populations away from the original sites of introduction.

However, species often sit along a continuum that spans these three stages (Richardson & Pyšek 2012) and this classification doesn't account for species abundance or impact in new locations, and thus the extent to which species have successfully overcome biotic and abiotic barriers to establishment. A species may successfully spread away from introduction sites into the wild but may still fail to become fully invasive if it cannot persist in those new locations for extended periods of time (Blackburn *et al.* 2011). To differentiate between species at different stages of the continuum between naturalisation and invasion, in this thesis I focus on species' classification within New Zealand, which takes into account their degree of spread and abundance (Howell & Sawyer 2006; Howell 2008):

Casual	Reproducing only in the immediate vicinity of the cultivated parent plant, or widespread but only known as a few isolated individuals.
Naturalised	Species form a self-sustaining population or occur repeatedly in natural or semi natural habitats or urban environments.
Invasive	I consider a plant species invasive if it is classified as an environmental weed in New Zealand. The species is reported as having a significant effect on at least one site designated as conservation land, indicating it has successfully established and spread beyond introduction sites.

1.4.3 The study species

In 2009 I identified populations of all the *Acacia* recorded as naturalised in New Zealand (Table 1.1) that were present within a 2 hour drive of Christchurch by examining herbarium records and contacting staff at the Department of Conservation, Environment Canterbury and Christchurch City Council. During the 2009-2010 reproductive season I conducted preliminary observations of all species I located to identify focal species for further research. My aim was to select species that are all naturalised but vary in the degree to which they have established and spread in line with the three classifications I outlined above. I focused on species along the continuum from causal to invasive since the processes of reproduction, seed dispersal and establishment in new locations are tightly linked to species' progression through each of these stages.

Of the species that are classed as environmental weeds in New Zealand I selected *A. dealbata* for further study. *Acacia decurrens*, *A. longifolia*, *A.verticillata* and *A. paradoxa* were difficult to locate and not widespread within the study area. Although *A. melanoxylon* and *A. mearnsii* were widespread and abundant within the study area, *A. melanoxylon* trees often had tall straight stems making pollination studies difficult and *Acacia mearnsii* populations appeared highly susceptible to galling meaning seed set was low and seed dispersal experiments would not have been possible. Among species at different stages of naturalisation and invasion I selected *A. baileyana* and *A. pravissima*, based on the availability of populations or individuals within the study area and the similarity of floral morphology and seed dispersal syndromes (see below). The species have also shown differential success on a global scale, which correlates with their performance in New Zealand (Richardson & Rejmánek 2011).

Acacia dealbata Link is a widespread and common tree found throughout south-eastern Australia (Maslin 2001). In New Zealand it is considered invasive and is recorded as an environmental weed. It has spread to form extensive monocultures along agricultural margins and in riverbeds. This species is also a widespread invader globally, particularly in southern Africa, the Americas and Mediterranean Europe (Richardson & Rejmánek 2011) where it is again often seen invading riverbeds and roadsides (Le Maitre *et al.* 2011). In New Zealand *A. dealbata* was introduced for soil conservation and shelter (Shelbourne *et al.* 2000) and has been planted or trialled for small-scale timber production throughout New Zealand.

Acacia baileyana F. Muell. is a small tree or shrub that is native to a small area around Cootamundra in New South Wales where it occurs in the forest understorey or in forest gaps (Maslin 2001), although it has been widely planted and is now naturalised beyond this range in Australia (Figure 1.2). *Acacia baileyana* was introduced to New Zealand for ornamental purposes and has been widely planted, though at low densities. It has recently been noted as invasive in New Zealand (Richardson & Rejmánek 2011). However, although widely naturalised it rarely spreads far from source populations, with only a few naturalised trees present at any locality. *Acacia baileyana* is considered invasive in Africa (Richardson & Rejmánek 2011).

Table 1.1 Naturalised species of *Acacia* in New Zealand, their introduction date, native range and invasive status. If introduction dates are not known * indicates the date naturalized (Webb *et al.* 1988). NSW: New South Wales; ACT: Australian Capital Territory; Qld: Queensland; Vic: Victoria; SA: South Australia; Tas: Tasmania; NT: Northern Territory. Species included in this thesis are highlighted in bold.

species	**introduction date/first record**	**native range**	**status in NZ(1)(2)**	**invasive elsewhere?(6)**
Acacia baileyana	**1916(3)**	**NSW**	**Fully naturalised**	**yes**
Acacia dealbata	**1868(3)**	**NSW & ACT**	**Environmental weed**	**yes**
Acacia decurrens	1851(3)	NSW & ACT	Environmental weed	yes
Acacia elata	1981*	NSW	Fully naturalised	yes
Acacia floribunda	1912(3)	Qld, NSW, Vic	Fully naturalised	no
Acacia longifolia	1863(3)	Qld, NSW, Vic, SA, Tas	Environmental weed	yes
Acacia maidenii	1911(3)	Qld, NSW, Vic	Casual	no
Acacia mearnsii	1871(3)	Qld, NSW, Vic, SA, Tas	Environmental weed	yes
Acacia melanoxylon	1870(3)	Qld, NSW, Vic, SA, Tas, ACT	Environmental weed	yes
Acacia paradoxa	1874(3)	Qld, NSW, SA, Tas, ACT	Environmental weed	yes
Acacia parramattensis	1959(4)	ACT, NSW	Fully naturalised	no
Acacia podalyriifolia	1981*	NSW	Casual	yes
Acacia pravissima	**1985(5)** †	**ACT, NSW, Vic**	**Casual**	**no**
Acacia saligna	1912(1)	NSW, NT, Qld, SA, Vic	Casual	yes
Acacia sophorae‡			Environmental weed	
Acacia stricta	1988*	NSW, Qld, SA, Vic, Tas	Casual	yes
Acacia verticillata	1922(1)	NSW, SA, Tas, Vic	Environmental weed	yes

† earliest record but noted as widely planted as an ornamental at this time and first mention of it in New Zealand 1924

‡ many authors regard this species as synonymous with or a subspecies of *A. longifolia*

(1) (Howell & Sawyer 2006)

(2) (Howell 2008)

(3) (Papers Past New Zealand 2012)

(4) (Webb *et al.* 1988)

(5) (Sheppard 1987)

(6) (Richardson & Rejmánek 2011)

Acacia pravissima F. Muell. ex Benth is a small tree or shrub native to higher elevation zones of the Australian Great Dividing Range where it is found in open eucalypt forest and moist areas (Maslin 2001). In New Zealand this species has a similar planting history to *A. baileyana*. It was introduced for ornamental purposes and is now widely planted, though at low densities. It can reproduce successfully but has not spread beyond garden plantings and is currently classed as a casual (Howell & Sawyer 2006). There are no records of it being invasive elsewhere in the world (Richardson & Rejmánek 2011). This species is morphologically distinct from the other two species in that it has phyllodinous leaves, rather than the bipinnate leaves of *A. dealbata* and *A. baileyana* (Figure 1.2).

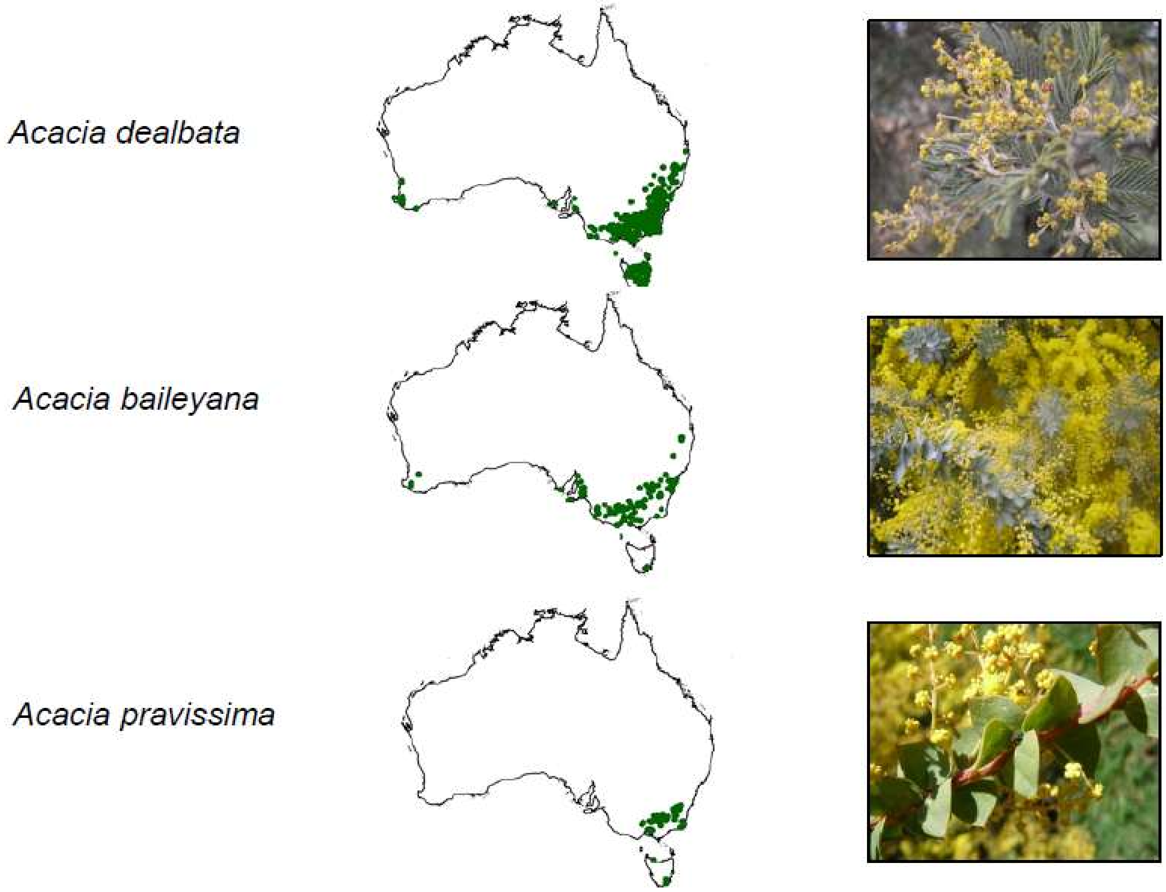

Figure 1.2 The three species included in this study, their distribution within Australia (data derived from an edited version of the Australian Virtual Herbarium; Council of Heads of Australasian Herbaria 2012) and an example of their leaves and flower heads. *Acacia dealbata* and *Acacia baileyana* exhibit bipinnate leaf forms where leaves are divided into pairs of pinnae, which are further subdivided into pinnules. *Acacia pravissima* has phyllodinous leaves where leaves are technically absent and replaced with enlarged photosynthetically active pyhllodes. Note that the maps also include records of species outside their native range in Australia.

1.5 The role of mutualisms in the invasion success of *Acacia*

A number of factors have been implicated in the success of alien *Acacia*, in particular traits associated with reproduction (Gibson *et al.* 2011). However, in contrast to many successful invaders, *Acacia* are reliant on mutualistic interactions for seed set and dispersal, as well as the establishment of symbiotic associations with nitrogen-fixing bacteria (rhizobia). Therefore, if mutualistic interactions are an important determinant of invasion success, species showing variable invasion outcomes where introduced should differ in the extent to which they are able to form effective mutualisms in new locations. However, no studies have yet quantified the strength of interactions in the native and introduced range of species that vary in invasiveness.

1.5.1 The reproductive ecology of *Acacia*

Acacia are adapted for generalist pollination and produce large numbers of brightly coloured often strongly scented flowers (Figure 1.2). Flowers are grouped into inflorescences that are either globose or spicate and are in turn arranged on racemes. Pollen is presented on the surface of the flower heads, which means that no specialist adaptations for pollination are required (Stone *et al.* 2003) and any insect or bird moving through the canopy could potentially act as a pollinator. Species do not produce floral nectar, and those that do secrete it through glands known as extra-floral nectaries that are present on the petiole and rachis of leaves. Insects, in particular bees, are more commonly associated with *Acacia* pollination (Bernhardt 1987; Grant *et al.* 1994; Stone *et al.* 2003), but there is some evidence that birds are attracted to plants by the extra-floral nectaries and may therefore also be involved (Ford *et al.* 1979; Vanstone & Paton 1988).

The majority of *Acacia* studied appear to be self-incompatible (Kenrick & Knox 1989; Grant *et al.* 1994; Morgan *et al.* 2002; Broadhurst *et al.* 2008; Gibson *et al.* 2011), though the degree of self-incompatibility varies between species (Kenrick & Knox 1989; Gibson *et al.* 2011). There is some evidence that *A. dealbata* introduced to South Africa is self-compatible (Rodger 2011). In all *Acacia* the stigma is receptive before pollen is released, which may be a mechanism to prevent self-fertilisation (Kenrick 2003). The timing of flowering in *Acacia* may be important for their reproductive success, since flowers are long-lived and open asynchronously both within individuals and within flowerheads (Kenrick 2003; Stone *et al.* 2003). Where *Acacia* species occur together there is evidence they will co-flower, which has the benefit of making them highly attractive to the available pool of pollinators, but the disadvantage of increasing competition between congeners and increasing the risk of pollen blocking by the deposition of heterospecific pollen (Bernhardt & Walker 1984; Stone *et al.* 2003). The combination of high floral rewards and floral longevity is predicted to enhance the competitive ability of *Acacia* in new locations and facilitate successful reproduction (Gibson *et al.* 2011).

Despite detailed knowledge of the reproductive ecology of *Acacia* in their native range, there are few studies that directly quantify overall reproductive success for different species within Australia (Moncur *et al.* 1991; Cunningham 2000; Broadhurst & Young 2006), and only one study that has examined *Acacia* reproduction outside their native range (Rodger 2011). Therefore, although their generalist pollination syndrome and ability to produce large quantities of viable seed is implicated in their invasion success, this has not been directly tested. In addition, reproduction in *Acacia* may be particularly vulnerable to the actions of natural enemies, such as plant pathogens and pre-dispersal seed predators (Auld 1986b; Impson & Moran 2004). For this reason, any decrease in pollination success may be mitigated by escape from natural enemies yet no studies of the reproductive success of alien *Acacia* take the potentially mediating role of these natural enemies into account.

1.5.2 *Acacia* and seed dispersal

It is generally accepted that *Acacia* are adapted for dispersal by birds and ants (Davidson & Morton 1984), though some dispersal by wind or water may occur. Davidson & Morton (1984) examined morphological and chemical characteristics of 20 species of Australian *Acacia* in relation to their dominant dispersal syndrome and found that species primarily distributed by birds had brightly coloured red, orange or yellow arils with seeds that remain hanging from the tree for longer, while those dispersed only by ants had smaller white arils. While birds are likely more important for long distance dispersal events, benefits of myrmecochory thought to be particularly important include directed dispersal into suitable sites for germination and vertical movement into the soil seed bank (Giladi 2006). Such vertical movement not only protects seeds from seed predation but is also considered important for the accumulation of large seedbanks that are implicated in the invasive success of alien *Acacia* (Holmes 1990; Richardson & Kluge 2008).

The three species I examine all have small white arils, indicating myrmecochory as the primary means of dispersal. While myrmecochory is considered relatively diffuse (Horvitz & Beattie 1980; Pemberton 1988), the ant fauna of New Zealand is depauperate and myrmecochory has not been reported in the native flora (Don 2007; Thorsen *et al.* 2009). However, if species do lose their dispersal mutualists, this may be compensated for by loss of seed predators, since many species of ant act as both seed dispersers and seed predators (Hughes & Westoby 1990). Although seed dispersal in *Acacia* is relatively well-studied in Australia (Andersen & Ashton 1985; Hughes & Westoby 1990; Ireland & Andrew 1995), there are few studies that have examined it in the introduced range of *Acacia*, the exception being two that have examined the dispersal of the invasive *A. cyclops* and *A. saligna* in South Africa (Glyphis *et al.* 1981; Holmes 1990), and none that have examined it for non-invasive alien *Acacia*. Therefore, as with pollination, the potential role of seed dispersal in determining the invasive success of introduced *Acacia* remains unknown.

1.5.3 The role of rhizobia

Interactions between *Acacia* and rhizobia have been reasonably well studied within Australia (Roughley 1987; Barnet & Catt 1991; Thrall *et al.* 2000; Thrall *et al.* 2007) and the importance of some species for forestry means that interactions between *Acacia* and rhizobia have also been examined in their introduced range (Habish & Khairi 1970; Prin *et al.* 2003; Boukhatem *et al.* 2012). In addition, there has been a recent increase in research focused on interactions between invasive *Acacia* and rhizobia (Rodríguez-Echeverría *et al.* 2011; Birnbaum *et al.* 2012), and *Acacia* are becoming a model system with which to examine the extent to which rhizobia might influence plant invasion.

All *Acacia* examined have been recorded to nodulate with rhizobia, predominantly species within the genus *Bradyrhizobium* (Rodríguez-Echeverría *et al.* 2011) though recent work suggests the rhizobia associated with *Acacia* in their native range is more diverse than previously thought (Hoque *et al.* 2011). There is evidence that compatible rhizobia are potentially widespread both within their native range and where introduced (Barnet & Catt 1991; Rodríguez-Echeverría *et al.* 2011). However, species vary in the extent to which they are able to form associations with the bacteria available, depending on the identity and density of strains present (Thrall *et al.* 2000; Thrall *et al.* 2007). There are examples of them either failing to establish effective symbioses in new locations or showing reduced performance (Turk *et al.* 1993; Weir 2006), which may be because rhizobia are limiting for species when first introduced (Parker *et al.* 2006).

While the success of *Acacia* in South Africa has been attributed to the presence of native congeners and their associated rhizobia (Parker 2001), New Zealand lacks any native *Acacia*. However, species of *Bradyrhizobium* are present in New Zealand, where they have been recorded to nodulate with exotic plant species, including the invasive *A. longifolia* (Weir *et al.* 2004; Weir 2006). In contrast, native species in New Zealand predominantly nodulate with species of *Mesorhizobium* and have not been recorded nodulating with *Bradyrhizobium* (Weir *et al.* 2004; Weir 2006). A considerable gap in our knowledge is the extent to which compatible rhizobia are available for all species in new locations, and whether this underpins the variable success of introduced *Acacia*.

1.6 Thesis objectives

I focus on each of the stages previously outlined to determine whether or not mutualistic interactions can explain the variable invasion success shown by *Acacia* introduced to New Zealand. At each stage I quantify whether the strength of interactions varies for species in the introduced relative to the native range, then examine whether this varies between species that have shown differential success since introduction. This is the first study to directly quantify the strength of biotic interactions for alien plant species showing differential invasive success in their native and introduced range. This research will not only provide important insights into the invasion process of a globally important group of invaders, but also improve our understanding of the role of biotic interactions in the invasion process in general. Broadly, I ask:

1. Does the strength of mutualistic interactions and the mediating role of antagonistic interactions vary for species in their introduced New Zealand relative to their native Australia range (is there a country effect)?
2. Does the extent to which the strength of interactions varies in the introduced relative to the native range vary for species that have shown differential success since introduction (is there a species × country interaction)?

Specifically, I apply these questions to the three stages outlined above and ask:

3. Seed set: are there differences in the reproductive success and influence of predispersal seed predation for species in New Zealand, relative to Australia, and does this correlate with how widespread the species is in New Zealand?
4. Seed dispersal: does the strength of seed dispersal and predation following seed fall vary for species in New Zealand, relative to Australia, and does this correlate with how widespread the species is in New Zealand?
5. Seedling performance: is the performance of species introduced to new locations in New Zealand limited by the availability of rhizobia, relative to their native range in Australia, and does this correlate with how widespread the species is in New Zealand?

1.7 Thesis outline

Chapters 2 - 4 have been written as self-contained research papers meaning there is some repetition in the introduction and methods sections. Each of the data chapters deals with each stage I have previously outlined and all are based on original research conducted in New Zealand and Australia between 2009 and 2012. Chapter 5 synthesises the results from chapters 2 - 4 and discusses the implications of the findings within the thesis as a whole.

Chapter 2 - Pollinators and predators at home and away: do they determine invasion success for *Acacia* in New Zealand?

2.1 Abstract

1. Introduced plant species are expected to benefit from escaping their natural enemies, but can also leave behind necessary mutualists. Since interactions with pollinators and pre-dispersal seed predators are important determinants of reproductive output, they are often implicated in alien plant success. Here I quantify the strength of these interactions in both the native and introduced ranges of three species of *Acacia* to determine if they can explain variation in invasion success.
2. I measured pods per inflorescence, rates of seed abortion and mean seed weight as indicators of pollination success, and determined losses to pre-dispersal insect and bird seed predators in both the native Australian and introduced New Zealand ranges. I also measured overall seed output for each species in each range. I predicted that if interactions with either pollinators or pre-dispersal seed predators were important for *Acacia* invasion then variation in their reproductive success and overall seed output should correlate with observed differences in the rates of establishment and spread of these species in New Zealand.
3. I found that pods per inflorescence and seed abortion rates were similar among the three species, and between the native and introduced ranges. In addition, for *A. dealbata*, a highly invasive species, and *A. baileyana*, a species that is widely naturalised in New Zealand, I found no differences in seed weight, pre-dispersal seed predation rate or overall seed output. However, for *A. pravissima*, currently considered a casual species in New Zealand, pre-dispersal seed predation rate was lower in New Zealand and overall seed production was much higher relative to conspecifics in Australia, and relative to the other two species.
4. These findings imply that neither mutualistic interactions with pollinators nor antagonistic interactions with pre-dispersal seed predators can explain differences among *Acacia* species in their invasion success. In addition, factors other than pollination success must explain the high reproductive output shown by *A. pravissima* in New Zealand, and variation in reproductive output cannot account for the relative success of the three species.

2.2 Introduction

In plants, prolific reproduction is frequently associated with invasion success (Rejmánek 1996; Pyšek & Richardson 2007). Interactions that influence seed output, such as those with pollinators and predispersal seed predators, may therefore be of particular importance for the establishment and spread of alien plant species (Baker 1974; Pyšek & Richardson 2007). The potential for interactions with predispersal seed predators to promote or hinder plant invasion has long been recognised (Rees & Paynter 1997; Fenner & Lee 2001; Wolfe 2002), and there is an increasing focus on the extent to which mutualistic interactions with pollinators might influence plant invasion (Richardson *et al.* 2000a; Parker & Haubensak 2002; Stout 2007; Vanparys *et al.* 2008). Current evidence suggests that, because most plant species have a generalised pollination syndrome (Waser *et al.* 1996), most introduced plant species should readily encounter pollinators in new locations (Richardson *et al.* 2000a; Stout *et al.* 2006; Gibson *et al.* 2011). Introduced plants may also benefit by leaving behind specialist natural enemies that reduce seed output. However, despite the potential advantage of losing enemies whilst retaining pollinators, the majority of introduced plant species do not become invasive. It is not known whether the degree to which introduced species have maintained or lost interactions with pollinators and predispersal seed predators can explain the variation in invasion success among alien plant species.

Successful pollination depends on both the quantity and quality of pollination events (Aizen & Harder 2007). The generalised nature of many pollination mutualisms, together with the widespread introduction of many generalist pollinators (Goulson 2003; Hanley & Goulson 2003), means that most introduced plant species are expected to experience sufficient quantity of pollination events (Baker 1974; Stout *et al.* 2006; Abe *et al.* 2011). However, the effectiveness of new associations can be highly context specific and vary according to the landscape into which species are introduced and the behaviour of pollinators (Grant *et al.* 1994; Parker 1997; Brown & Mitchell 2001). For example, in a landscape with high plant species diversity those species that attract generalist pollinators may be susceptible to pollination failure resulting from the blocking of pollination by heterospecific pollen deposition (Brown & Mitchell 2001; Stone *et al.* 2003), while the large floral rewards that often facilitate generalist pollination provide little incentive for between plant movements and can reduce the likelihood of pollen transfer between individuals (Charlesworth & Charlesworth 1987; Grant *et al.* 1994; Stone *et al.* 2003).

Currently, most research on the role of pollination mutualisms in plant invasion has focused on identifying reproductive traits that can facilitate effective pollination. Most notably, high self-compatibility is identified as important since it reduces the reliance on between plant pollen transfer (van Kleunen & Johnson 2007; Burns *et al.* 2011; Hao *et al.* 2011) and a number of field studies have confirmed the prevalence of self-compatibility in invasive plants when compared to native or non-invasive species (Powell *et al.* 2011; Petanidou *et al.* 2012; Ward *et al.* 2012), or in the introduced versus the native range (Petanidou *et al.* 2012). However, it is overall reproductive success and seed output that is likely to drive invasion outcomes, of which pollination success is just one component. Therefore, quantifying variation in overall reproductive success, both among alien plant species and between their native and introduced ranges, might better explain invasion outcomes and provide the context within which to understand the importance of components such as pollen limitation and pre-dispersal seed predation.

While pollination mutualisms are more frequently generalist, predispersal seed predators are often quite specialist and appear much less common in exotic plant populations (Memmott *et al.* 2000; Wolfe 2002; Liu & Stiling 2006). It is suggested that escape from predispersal seed predators may mask the negative effects of pollen limitation (Kéry *et al.* 2001; Vaupel & Matthies 2012), since it could mitigate any reduction in seed output associated with lower pollination success. There is also evidence that where predispersal seed predators are present, population recruitment is limited, which forms the basis of many biocontrol programmes (Louda 1983; Rees & Paynter 1997; Impson & Moran 2004). For these reasons, demonstrating a role of pollen limitation in the failure of plant species to invade also requires quantifying the potential influence of predispersal seed predators. Here, I aim to assess the extent to which both pollination and predispersal seed predation may influence plant invasion by examining their contribution to reproductive success and overall seed output in the native and introduced range of three alien species that have shown varying invasion outcomes. I studied Australian species within the genus *Acacia* that have been introduced to New Zealand.

The Australian *Acacia* are a large group of trees and shrubs that have been widely introduced around the world for forestry and horticulture. Where introduced, a relatively large proportion (around 6%) have succeeded at establishing and becoming invasive (Richardson *et al.* 2011). Traits associated with pollination are one factor identified as potentially contributing to their success (Gibson *et al.* 2011) and all species within this group share a generalist pollination syndrome and produce large quantities of brightly coloured yellow flowers (Stone *et al.* 2003; Gibson *et al.* 2011). However, many species are also self-incompatible (Kenrick & Knox 1989), which may limit the effectiveness of pollination in new locations and there is evidence that species differ in their attractiveness to pollinators present (Bernhardt & Walker 1984). As current research on the pollination ecology of *Acacia* outside Australia is limited, it is not known whether reproductive failure is the reason not all species become invasive when introduced. In addition, the reproductive success of Australian *Acacia* can be strongly influenced by interactions with natural enemies, for example gall-forming fungi and wasps (Dennill & Donnelly 1991; Adair *et al.* 2009; Wingfield *et al.* 2011) and seed feeding insects (Dennill & Donnelly 1991), which is highlighted by the success of the biological control of some *Acacia* species in South Africa (Dennill *et al.* 1999; Hill *et al.* 2000; Impson *et al.* 2011; Wingfield *et al.* 2011). Again, no studies that have examined introduced *Acacia* have considered both positive and negative influences on reproductive output.

At least 150 species of Australian *Acacia* have been introduced to New Zealand (Appendix A; Diez *et al.* 2009), of which 17 have naturalised, defined as having established self-sustaining populations (Howell & Sawyer 2006) and eight are sufficiently widespread to be classed as environmental weeds (Howell 2008). In their native range *Acacia* are pollinated by a variety of flies, beetles and bees, though some bird pollination may also occur (Bernhardt *et al.* 1984; Bernhardt 1987; Vanstone & Paton 1988; Moncur *et al.* 1991). Little is known of the pollination of *Acacia* in New Zealand, but much of the native New Zealand flora shows generalist pollination by a range of insects (Heine 1937; Newstrom & Robertson 2005), including the introduced European honeybee *Apis mellifera* Linneaus, which also pollinates *Acacia* species in Australia (Bernhardt 1987; Moncur *et al.* 1991) and where introduced in South Africa (Rodger 2011). Birds may also be involved in pollination, particularly during mid-late winter when many *Acacia* species flower and insect activity is reduced (Ford *et al.* 1979). In New Zealand the natural enemies of *Acacia* comprise a range of species that have also been introduced from species' native ranges in Australia, though their means of introduction are unknown. Those present include the gall forming *Uromycladium* fungus, the seed feeding wasp *Bruchophagus acacia*e Cameron, psyllids (Dick 1985; Appleton *et al.* 1997; Hill *et al.* 2000) and a variety of other pests.

I measured the relative reproductive success, determined by the success of pollination, and the strength of predispersal seed predation for three introduced *Acacia* species in New Zealand and compared this to their native range in Australia. I also quantified total seed output measured as seed rain per m^2. If interactions with pollinators and predispersal seed predators are important in *Acacia* invasion then variation in the reproductive success and overall seed output should be linked to differences in the rates of establishment and spread since introduction to New Zealand. I asked two questions:

1. Are there differences in reproductive success of *Acacia* as a result of pollination limitation or predispersal seed predation between species in New Zealand, relative to Australia?
2. Can differences in reproductive output explain the differential invasion success of *Acacia* species in New Zealand?

2.3 Methods

I selected three species that differed in the degree to which they have naturalised and spread in New Zealand (Table 2.1) but are all native to temperate regions of south-eastern Australia. The three species all flower in mid-late winter and produce pods in early to mid-summer, with some overlap between all species (Table 2.1). As with all other *Acacia*, species produce large quantities of brightly coloured flowers. Flowers are extremely small and grouped together in globular flower heads (inflorescences) that are arranged into racemes (Bernhardt *et al.* 1984). The number of inflorescences per raceme can be highly variable both between and within species. Per capita flower production is extremely high and seed set, while often high in absolute terms, is low relative to flower production (Moncur *et al.* 1991; Morgan *et al.* 2002). Pollen is grouped together into a polyad and polyad grain number is approximately equal to or more than ovule number (Table 2.1; Moncur *et al.* 1991; Grant *et al.* 1994). This has the advantage of ensuring the pollination of all ovules following pollen deposition but the disadvantage of giving each flower only one chance at pollination, meaning that the potential for pollen blocking is high (Moncur *et al.* 1991). Within flowers, the stigma is generally receptive before pollen is released, providing a mechanism to reduce self-fertilisation (Moncur *et al.* 1991). Flowers do not produce nectar but plants secrete it through glands known as extra-floral nectaries, which are located on the rachis in the case of *A. dealbata* and *A. baileyana* but may also be found on the phyllodes of *A. pravissima*. The floral morphology of species means that any animals moving about in the canopy of plants could potentially act as pollinators (Stone *et al.* 2003; Gibson *et al.* 2011).

Acacia are vulnerable to predispersal seed predation by a range of insects, most notably seed feeding weevils within the genus *Melanterius*, and seed feeding wasps in the genus *Bruchophagus* (New 1983; Auld 1986b; Hill *et al.* 2000). Insects oviposit on the seeds while they are green and immature and larval development is completed within the seeds. There is little information on the importance of predispersal seed predation by birds for *Acacia*, but it has been recorded in Australia (Whitney 2005) and pilot studies in New Zealand in the 2009-2010 reproductive season recorded some seed destruction by birds. The three species are also susceptible to a variety of gall forming wasps, rusts and midges, stem borers and psyllids (New 1983; Auld 1986b; Dennill & Donnelly 1991; Appleton *et al.* 1997; Adair *et al.* 2000; Impson & Moran 2004; Adair *et al.* 2009; Wingfield *et al.* 2011). Although natural enemies appear more frequently recorded on *A. dealbata* and *A. baileyana* in both the native and introduced range than on *A. pravissima*, information on *A. pravissima* is sparse in general.

Acacia dealbata Link is widespread and common throughout south-eastern Australia (Maslin 2001) and is invasive in New Zealand, where it has spread to form extensive monocultures along agricultural margins and in riverbeds. This species is also invasive in other parts of the world, particularly southern Africa, the Americas and Mediterranean Europe (Richardson & Rejmánek 2011). It appears to have a mixed reproduction strategy, with evidence of both self-compatibility (Gibson *et al.* 2011; Rodger 2011), and self-incompatibility (Broadhurst *et al.* 2008). When self-fertilisation is successful, inbreeding depression (Charlesworth & Charlesworth 1987) can occur and progeny often show reduced fitness (Rodger 2011). This species can also reproduce vegetatively (Maslin 2001). *Acacia baileyana* F. Muell. is native to a small area around Cootamundra in New South Wales where it occurs in the forest understorey or in forest gaps (Maslin 2001), although it has been widely planted and is now naturalised beyond this range in Australia. In New Zealand, *A. baileyana* is a popular amenity tree and although widely naturalised it rarely spreads far from source populations, with only a few naturalised trees present at any locality. *Acacia baileyana* is considered invasive in Africa (Richardson & Rejmánek 2011). In common with most *Acacia* studied, this species is highly self-incompatible

Table 2.1 Invasion status, breeding system, phenology and key reproductive traits for the three species of *Acacia* included in this study: *Acacia baileyana*, *Acacia dealbata*, *Acacia pravissima*.

species	**introduction date***	**invasion status(1)(2)**	**self-compatible?**	**flowering(3)**	**seed set(3)(4)**	**ovule no.(5)**	**polyad grain no.(5)**	**seed mass(6)(7)**
A. dealbata	1868(8)	invasive	partially(7, 10)	July-Nov	Dec-Mar	12	16	9.1 - 12.2 mg
A. baileyana	1916(8)	naturalised	no(11)	June-Aug	Oct-Jan	12	16	19.2 - 21.8 mg
A. pravissima	1985(9) †	casual	unknown	Aug-Nov	Jan-Mar	unknown	12 or 16(12)	7.2 - 8.4 mg

* Date of earliest record found
† Earliest record but noted as widely planted as an ornamental at this time
(1) (Howell & Sawyer 2006)
(2) (Howell 2008)
(3) (Maslin 2001)
(4) (Gunn 2001)
(5) (Kenrick & Knox 1982)
(6) (O'Dowd & Gill 1986)
(7) (Gibson *et al.* 2011)
(8) (Papers Past New Zealand 2012)
(9) (Sheppard 1987)
(10) (Rodger 2011)
(11) (Morgan *et al.* 2002)
(12) (Australasian Pollen and Spore Atlas 2013)

(Kenrick & Knox 1989; Morgan *et al.* 2002). When self-fertilisation does occur, the number of seeds per pod is reduced and rates of seed abortion increase (Morgan *et al.* 2002). Self-fertilisation under natural conditions appears to be high (Morgan *et al.* 2002). *Acacia pravissima* F. Muell. ex Benth is native to higher elevation zones of the Australian Great Dividing Range where it is found in open eucalypt forest and moist areas (Maslin 2001). In New Zealand it can reproduce but has not spread beyond garden plantings and is currently classed as a casual (Howell & Sawyer 2006).

2.3.1 Study sites

I carried out fieldwork for one reproductive season between May 2010 and February 2011. I selected up to five field sites per species in both Australia and New Zealand (Appendix B). In Australia, populations were located within the species' known native ranges (Maslin 2001), but for logistical reasons I limited searching for populations to within a three hour drive of Canberra (35°16'S 149°7'E). I first located *A. baileyana* and *A. pravissima* populations and then selected populations of the more widespread *A. dealbata* close to these sites (see Appendix B). In New Zealand study populations were located within a two hour drive of Christchurch (43°31'S 172°38'E). I included all *A. baileyana* and *A. pravissima* populations found with more than one individual (four of which contained fewer than five individuals), and five of the largest known *A. dealbata* populations (Appendix B).

2.3.2 Reproductive success

To measure the success of pod production I used the inflorescence as the unit of reproduction (Tybirk 1997; Broadhurst & Young 2006; Gibson *et al.* 2011) and quantified pods per inflorescence. I estimated four measures relating to reproductive success: a) the total number of racemes on a 30 cm length of branch on up to five branches per tree; and b) the total number of inflorescences on ten racemes; c) the number of racemes that had produced at least one pod on each branch; and d) the mean number of pods per raceme on ten racemes that had produced at least one pod. Measures a) and b) were estimated when flowers were at the yellow bud stage while c) and d) were undertaken when pods were mature and beginning to dehisce. I calculated pods per inflorescence using the following equation:

$$(c \times (d / 10)) / (a \times (b / 10)).$$

To measure the success of seed production I quantified the percentage of seeds initiated that were aborted per pod for each species in each country and the weight of seeds that were produced. I examined the percentage of seeds initiated that were aborted, rather than absolute numbers, to focus on relative differences in reproductive success between species and ranges and account for natural variation in seed production between the species. I haphazardly collected handfuls of pods from different locations on each focal tree. I then mixed these together and took a subsample of up to 30 pods from bulked samples. For each pod I recorded the number of seeds initiated, determined by the number of indentations in the seed pod (Morgan *et al.* 2002), and the number of these that were aborted. I weighed ten seeds from each tree to determine mean seed weight for each species in each country.

2.3.3 Estimate of natural enemy impacts

To determine whether natural enemies might disproportionally influence pod production in either range I recorded the presence or absence (0 or 1) of any obvious sign of natural enemies on each branch surveyed. I categorised them into gall forming, fungus or mould forming, and stem borers, then calculated an overall index for natural enemy attack by summing values for each to give a maximum level of natural enemy attack of 3 and a minimum of 0.

I measured predispersal seed loss to insects by counting the number of seeds in each pod that were infested, which was identified by the presence of frass on the seed pod and/or holes in the seed coat. I expressed pre-dispersal predation attributable to insects as a proportion of seeds matured that were infested.

I also determined whether predispersal seed predation by birds may influence seed output in either range. I set out four seed traps under the flowering portion of the canopy of each tree, with two close to the base and two towards the canopy edge. Traps were left out for the duration of seedfall in each species. Seed traps were constructed from a plastic bucket (diameter 25.3 cm) with holes drilled in the bottom to let rain water out and an inverted cone of fibre glass mesh (mesh size 1mm) glued to the top to catch seeds. A wire mesh lid (mesh size approximately 1cm) secured to the top of each trap protected seeds from further predation once caught, while metal pegs held traps in place. At the end of seed fall trap contents were bulked together to form one sample per tree and the total number of mature seeds in each trap and the total number of seed fragments or seeds that had been partially eaten were counted. Seeds that had not been fully formed were discounted.

2.3.4 Overall reproductive output

To examine overall seed production for each species in each range I determined the overall percentage of seeds initiated that were viable (number initiated – number aborted – number infested) and the total number of seeds falling as seed rain per m^2 (total number of whole, fully formed seeds in traps).

2.3.5 Statistical analysis

I had seven response variables (Table 2.2). I fitted linear mixed effect models to the data in R (R Development Core Team 2011) using the function lmer (library:lme4; Bates & Maechler 2012) and restricted maximum-likelihood methods (REML). This allowed me to include tree and/or site as random effects and fit data to a binomial distribution where appropriate (Table 2.2). To account for overdispersion in binomial data I included an overdispersion term to measure the degree to which the proportion of seeds either aborted, infested, viable or eaten for individual pods or trees deviated from that expected under a standard binomial distribution, conditional on the treatment and tree effects. I obtained mean values and associated confidence intervals for each variable I measured under each treatment combination, taking into account the nestedness of my experimental design, by creating a dummy variable coding for each combination of country and species and including any necessary overdispersion terms and random effects.

In order to determine which explanatory variables were important in explaining variation in the response data I used information-theoretic techniques (Burnham & Anderson 2004). I first identified a maximum model that contained all main effect and interaction terms of interest, and then constructed a candidate set of models using all subsets of this maximum model (Table 2.2). Relevant random effects and overdispersion terms were included in all models. To provide a measure of model fit I used the small sample version of Akaike's information criterion to allow for small samples sizes under some combinations of tree and site (AICc; Burnham & Anderson 2004). Smaller values of AIC_C indicate a better fitting model. I calculated delta AIC_C (ΔAIC_C), the difference between a model and the best fitting model in the candidate set, to determine the support for each model. The best fitting model has an ΔAIC_C of 0 and, in general, models that have an ΔAIC_C of ≤ 2 have

Table 2.2 Summary of the seven models fitted to the data, including the response variable, source of the data, the maximum model fitted, distribution used, whether or not an overdispersion term was fitted and the random effects included in the model

response variable	data source	explanatory variables (full model)	distribution	over-dispersion	random effects
reproductive success					
pods inflorescence^{-1}	5 branches tree^{-1}	species × country + natural enemies	normal	no	site:tree
percentage of seeds aborted	30 pods tree^{-1}	species × country	binomial	yes	site:tree
seed weight	10 seeds tree^{-1}	species × country	normal	no	site:tree
seed predation					
percentage of seeds infested	30 pods tree^{-1}	species × country	binomial	yes	site:tree
percentage of seeds eaten	4 seed traps tree^{-1} (bulked)	species × country	binomial	yes	site
overall reproductive output					
percentage viable seeds	30 pods tree^{-1}	species × country	binomial	yes	site:tree
total seed rain m^{2}	4 seed traps tree^{-1} (bulked)	species × country	normal	no	site

.

substantial support, those in which ≤ 4 $\Delta AIC_C \leq 7$ have weaker support and those with $\Delta AIC_C > 10$ have little support (Burnham & Anderson 2004). I often encountered situations where one or more models had a ΔAIC_C of ≤ 2 and models differed from each other only in the inclusion or exclusion of a single parameter. In these situations the inclusion of the extra parameter is adding little to the model fit (as measured by the log-likelihood) and so I identified the best-fitting model as the one involving the least number of parameters. I calculated AIC_C, ΔAIC_C, and the number of parameters in each model using the AIC_Cmodavg package (Mazerolle 2012).

2.4 Results

2.4.1 Reproductive success

Reproductive success measured as pods per inflorescence was low in both Australia and New Zealand with an overall mean of 0.06 (Figure 2.1a). There was no indication that the mean number of pods per inflorescence differed significantly between New Zealand and Australia or among the three species, since the model that included only an intercept term was among the most strongly supported ($AIC_C < 2$; Table 2.3a). There was also no indication that the level of natural enemy attack influenced pods per inflorescence. Despite a much higher percentage of branches in Australia showing evidence of galling, stem borers or fungus than New Zealand (Figure 2.1b), this did not appear to have a significant influence on pod production. In general, pods per inflorescence was highly variable with most of the variation (62.5%) occurring within individual trees (Table 2.1a). An exception to this was *A. dealbata* in Australia, which showed complete reproductive failure at two sites and the highest levels of pods per inflorescence (0.22) at a third (Appendix C).

Species varied in the percentage of seeds initiated that were aborted, with strong support for a model that included species as an explanatory variable (Table 2.3a). Abortion rates were lower in *A. pravissima*, with less than 25% of seeds initiated aborted, while *A. dealbata* and *A. baileyana* aborted 34% and 41% of seeds initiated, respectively (Figure 2.2a). Including country as an explanatory variable in the model did little to improve model fit, indicating that seed abortion rates did not differ appreciably between Australia and New Zealand

Seed masses also did not differ between New Zealand and Australia for any of the three species, since a model that included the species × country interaction was not much better supported than one that included only species as an explanatory variable (Table 2.3a). There was some indication that *A. pravissima* seeds were heavier in Australia, as seeds weighed around 3mg more than in New Zealand (10 mg compared to 7mg, respectively), but this was not significant.

2.4.2 Pre-dispersal seed predation

Overall, around 21% of seeds showed evidence of predispersal predation by insects. There was a strong species × country interaction on percentage seed infestation (Table 2.3b) that was due to *A. pravissima* experiencing almost no insect predation in New Zealand, where only 0.02% of seeds were damaged (Figure 2.3a). *Acacia pravissima* also experienced considerably lower insect seed predation in Australia than the other two species, with only 3% of its seeds damaged compared to 12% of *A. dealbata* and 28% of *A. baileyana* seeds. However, seed predation in general was highly variable and differences among species were only significant for *A. pravissima* and *A. baileyana* (Figure 2.3a).

Pre-dispersal seed predation by birds was lower than by insects with an average of 6% of seeds predated. Levels of bird predation differed among the three species and between Australia and New Zealand, with support for a model that included both species and country as explanatory variables (Table 2.3b). Mean seed predation by birds was around ten times higher in Australia than New Zealand (11% compared to around 1%, respectively), while more than twice as many seeds of *A. baileyana* were eaten by birds than the other two species (9% compared to 4% for both *A. dealbata* and *A. pravissima*).

2.4.3 Overall reproductive output

Species varied in the overall percentage of seed initiated that were viable once both seed abortion and seed predation had been taken into account (Table 2.3c). The percentage of seeds that were neither aborted nor predated was highest for *A. pravissima* (68%) compared to only 40% for *A. dealbata* and 33% for *A. baileyana* (Figure 2.4a). There was no indication that the percentage of viable seeds per pod varied between the two countries for any of the species examined, and this pattern was similar for the absolute numbers of seeds produced by each species in each country (Figure 2.4a). As with other variables examined, there was a large amount of variation, most of which occurred between sites

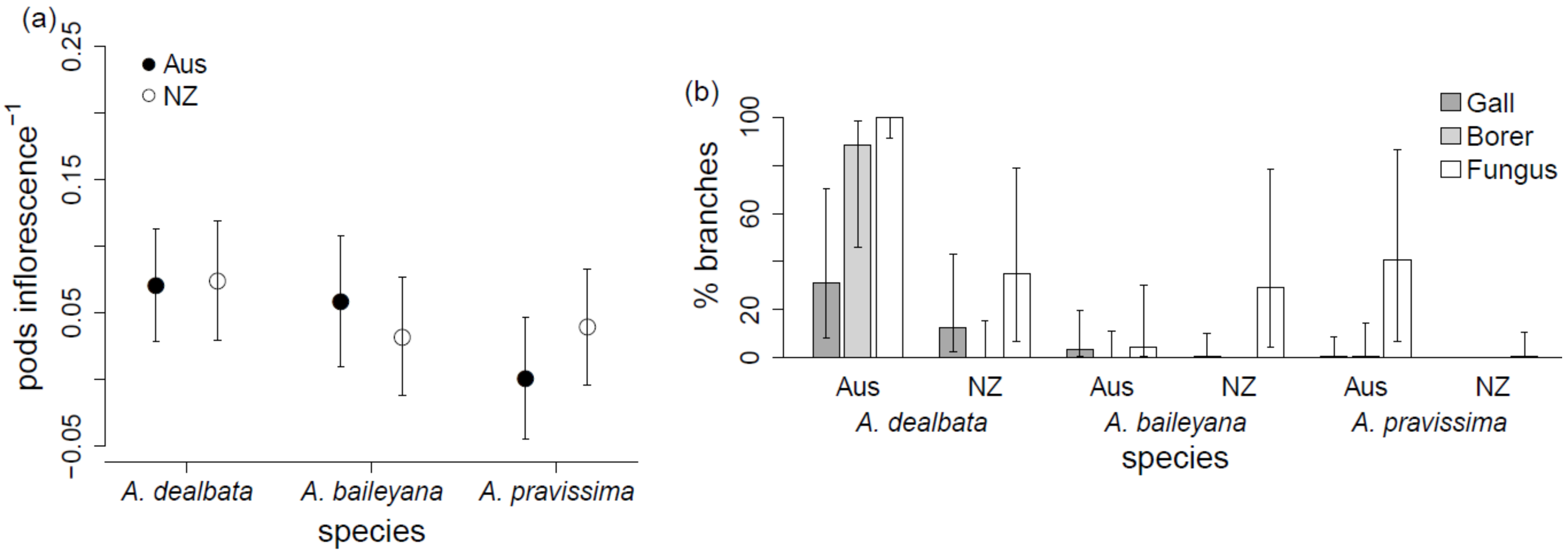

Figure 2.1 Reproductive success: pod production. Mean pods per inflorescence for three species of *Acacia* in their native Australia (Aus) range compared to their introduced New Zealand (NZ) range (a), and the percentage of branches showing signs of either galling, stem borers or fungus/moulds (b). Bars represent 95% confidence intervals.

Table 2.3 Comparison of linear mixed effect models for each of the response variables examined. k is the number of parameters in the model. AIC_C is the Akaike Information Criterion with correction for small sample size. ΔAIC_C shows the difference between the model AIC_C and the lowest AIC_C for the model set. The amount of variation accounted for by the random effects of trees within site, site and the variation that remained unexplained is given for the best model in each case (the one with ΔAIC_C values of ≤ 2 and the least number of parameters). Models with strong support are highlighted in bold.

model	**k**	**log likelihood**	**AICC**	**ΔAICC**	**% variation**		
					tree/site	**site**	**residual**
a) reproductive success							
pods inflorescence^{-1}							
species	**6**	**627.8**	**-1243.4**	**0**			
intercept	**4**	**625.1**	**-1242.1**	**1.3**	**24.5**	**13**	**62.5**
species + country	**7**	**627.8**	**-1241.4**	**2**			
enemies	5	625.7	-1241	2.4			
species + enemies	7	627.2	-1240.2	3.2			
country	5	625.1	-1239.9	3.5			
country + enemies	6	626	-1239.8	3.6			
species × country	9	629	-1239.6	3.8			
species + country + enemies	8	627.4	-1238.1	5.3			
species × country + enemies	10	629	-1237.2	6.2			
percentage of seeds aborted							
species	**6**	**-1875.2**	**3762.4**	**0**	**37**	**13.1**	**49.9**
species + country	**7**	**-1874.8**	**3763.7**	**1.3**			
species × country	**9**	**-1873**	**3764**	**1.6**			
intercept only	4	-1880.6	3769.2	6.9			
country	5	-1880.4	3770.8	8.4			

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model	k	log likelihood	AICC	ΔAICC	% variation tree/site	 site	 residual
seed weight							
species × country	**9**	**3218.5**	**-6418.7**	**0**			
species	**6**	**3214.4**	**-6416.7**	**1.9**	**58.9**	**0**	**41.1**
species + country	7	3214.9	-6415.6	3.1			
intercept only	4	3169.3	-6328.5	90.1			
country	5	3169	-6327.9	90.7			
b) pre-dispersal seed predation							
percentage eaten by insects							
species × country	**9**	**-1287.2**	**2592.5**	**0**	**31.9**	**31.9**	**35.9**
species	6	-1296.6	2605.2	12.8			
species + country	7	-1296.6	2607.2	14.7			
intercept only	4	-1320.3	2648.7	56.2			
country	5	-1320.3	2650.7	58.2			
percentage eaten by birds							
species + country	**7**	**-192.9**	**400.4**	**0**	**NA**	**69.8**	**30.2**
species × country	**9**	**-191.8**	**402.33**	**1.96**			
species	6	-200.5	413.4	13.04			
country	5	-203.7	417.75	17.38			
intercept	4	-210.3	428.8	28.4			

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model	k	log likelihood	AICC	ΔAICC	% variation		
					tree/site	site	residual
c) Overall reproductive output							
percentage of viable seeds pod^{-1}							
species	**6**	**-2018.5**	**4049**	**0**	**26.9**	**43.6**	**29.5**
species + country	**7**	**-2018.4**	**4050.9**	**1.9**			
species × country	9	-2016.8	4051.7	2.7			
intercept	4	-2027.6	4063.1	14.1			
country	5	-2027.5	4065.1	16.1			
overall seed rain (m^2)							
species × country	**8**	**-1005.7**	**2028.7**	**0**	**NA**	**26.5**	**73.5**
species + country	6	-1013	2038.8	10.1			
species	5	-1016.6	2043.7	15			
country	4	-1024.6	2057.3	28.7			
intercept	3	-1027	2060.2	31.6			

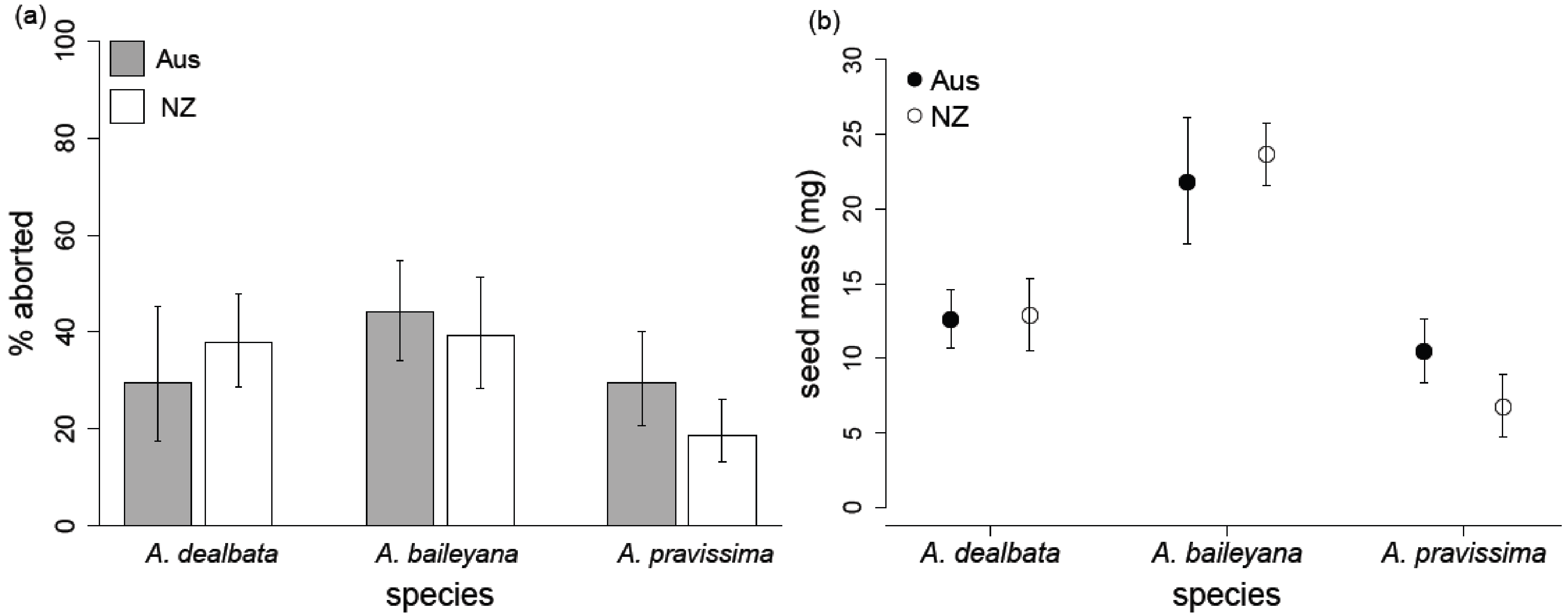

Figure 2.2 Pollination success: seed production. Percentage of seeds initiated that were aborted for three species of *Acacia* in their native Australia (Aus) range compared to their introduced New Zealand (NZ) range (a), and the mean weight of seeds produced (b). Bars represent 95% confidence intervals.

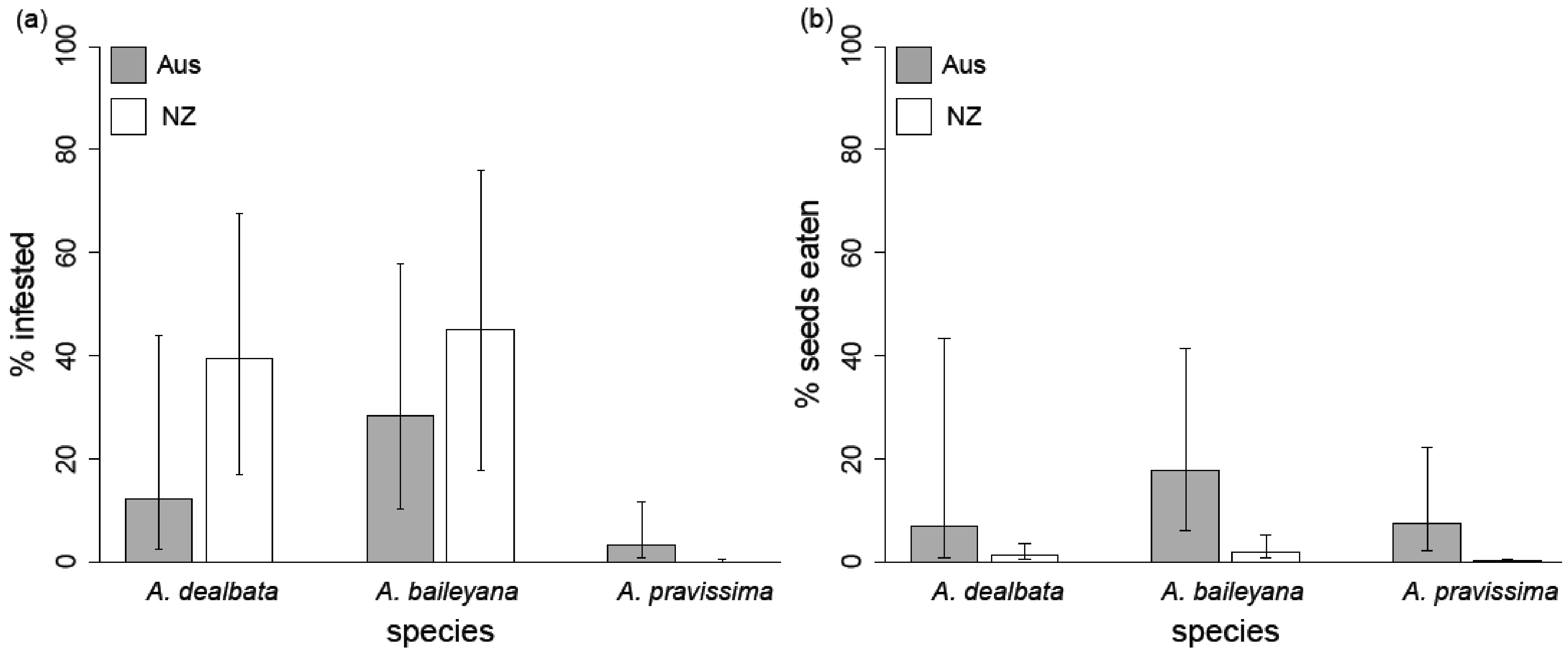

Figure 2.3 Pre-dispersal seed predation. The percentage of seeds that showed signs of insect infestation for three species of *Acacia* in their native Australia (Aus) range compared to their introduced New Zealand (NZ) range (a), and the percentage of seeds caught in traps that showed signs of bird predation (b). Bars represent 95% confidence intervals.

(43.6%; Table 2.3c). On a site by site basis, there was some indication that the combination of seed abortion and seed predation could considerably impact on the number of viable seeds produced for *A. dealbata* and *A. baileyana* (Appendix D).

Although pod production and overall seed production per pod did not differ for species in New Zealand compared to Australia, there was significant variation between species in the extent to which seed rain differed between the two countries (Figure 2.4b), with strong support for a model that included the interaction between species and country (Table 2.3c). Seed rain for *A. dealbata* and *A. baileyana* was similar in both Australia and New Zealand (Figure 2.4b). However, seed production per m^2 in *A. pravissima* was over ten times higher in New Zealand than Australia with trees producing more than 5000 seeds m^2 in New Zealand compared to less than 500 seeds m^2 in Australia. Since *A. pravissima* trees were also larger in New Zealand than in Australia (Appendix E), this difference likely reflects true variation in seed output between the two countries. For all three species seed rain was highly variable. Most variation occurred between individual trees (73.5%, Table 2.3c), but there were also differences between sites (Appendix F).

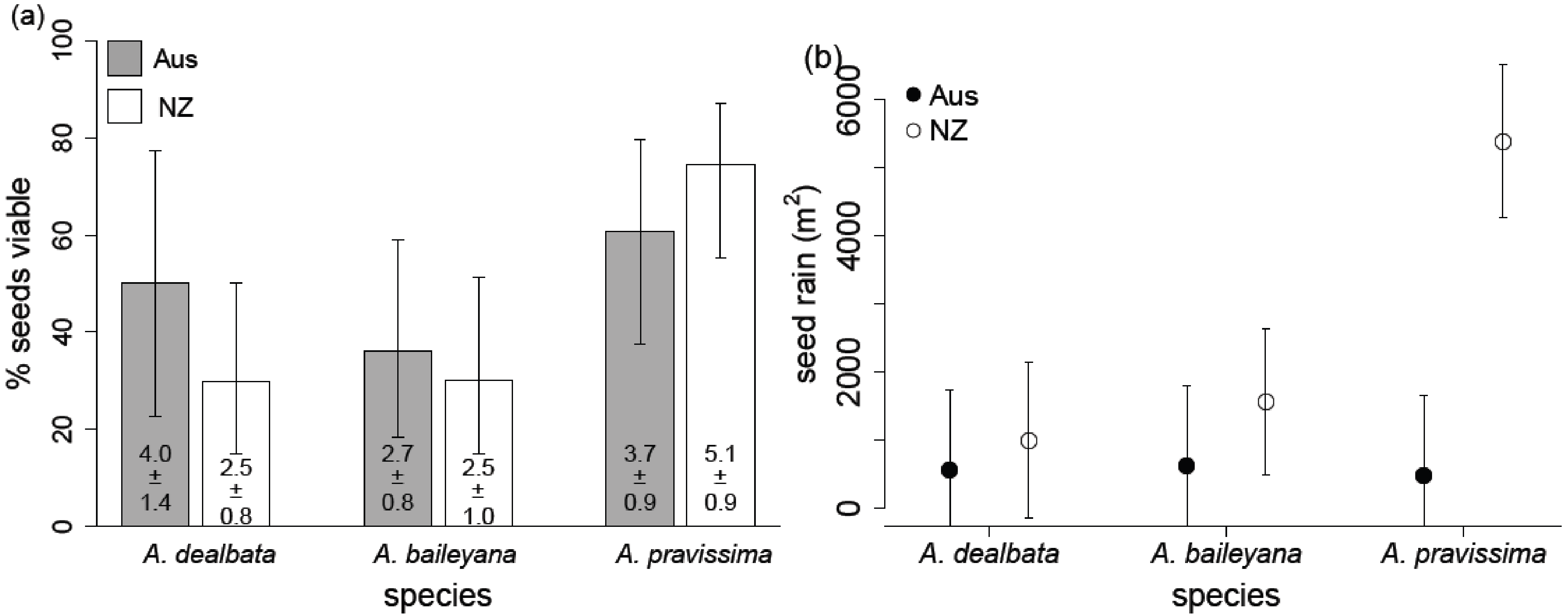

Figure 2.4 Overall reproductive output. The overall percentage of seeds initiated that were viable for three species of *Acacia* in their native Australia (Aus) range compared to their introduced New Zealand (NZ) range (a) and seed rain per m^2 (b). Bars represent 95% confidence intervals. Numbers indicate mean values ± 95% confidence intervals for actual number of seeds per pod.

2.5 Discussion

In this study the invasive *A. dealbata* and the widely naturalised *A. baileyana* did not appear to have any advantage or disadvantage in terms of interactions with pollinators or insect predispersal seed predators in New Zealand relative to Australia, though there was some reduction in bird seed predation. In addition, there was no indication that these interactions affected overall seed output (Table 2.4). In contrast, *A. pravissima*, which is currently considered as casual in New Zealand, demonstrated escape from all natural seed predators and a large increase in seed output in New Zealand compared to Australia and, in general, reproductive success was higher for this species (Table 2.4). These outcomes are not consistent with the hypothesis that interactions with pollinators and predispersal seed predators could explain differences in invasion success for these three *Acacia* species in New Zealand.

2.5.1 Reproductive success

The low pod production I found for all three species is consistent with those reported by other studies that also find limited reproductive success in *Acacia*, relative to investment. For example, a study of *A. dealbata* in its native range in Australia recorded between around 0.03 and 0.30 pods per inflorescence (Broadhurst & Young 2006), while the percentage of *A. baileyana* flowers producing pods in cultivated and weedy populations in Adelaide was found to vary from 0.07 to 0.41 (Morgan *et al.* 2002). Although no other studies have quantified reproductive success in *A. pravissima*, the frequency with which low pod production is reported in other species (Moncur *et al.* 1991; Brown *et al.* 2003) suggests that this is likely to be found throughout the genus. Similarly, the high rates of seed abortion I recorded also appear to be consistent with other studies of *Acacia*, with rates of up to 40% recorded in the same study of *A. baileyana* (Morgan *et al.* 2002) and of between 20 and 35% in studies of other Australian *Acacia* species (Brown *et al.* 2003).

Although species with a generalist pollination syndrome, such as *Acacia*, are expected to readily form pollination mutualisms in new locations (Gibson *et al.* 2011), for species that both produce large floral displays and show self-incompatibility the effectiveness of such associations may vary and the identity of the pollinating species could impact on their reproductive success (Charlesworth & Charlesworth 1987; Stone *et al.* 2003). However, that there was no difference in either pod production or seed abortion rates between the two ranges suggests that the reproductive success of *Acacia* introduced to New Zealand is

Table 2.4 Summary of species' differences in New Zealand relative to Australia (first symbol, in bold) and relative to each other (second symbol). 0, no difference; +, an increase in New Zealand relative to Australia or an increase relative to other species; -, a decrease in New Zealand relative to Australia or decrease relative to other species. Note that seed weights cannot be directly compared between the species and only the country effect or country × species interaction would be relevant.

species	**reproductive success**			**predispersal predation**		**overall output**	
	pods inflorescence^{-1}	seed abortion	seed weight	insect predation	bird predation	seeds pod^{-1}	seed rain
Acacia dealbata	**0** 0	**0** -	**0** 0	**0** 0	**+** +	**0** 0	**0** -
Acacia baileyana	**0** 0	**0** -	**0** 0	**0** -	**+** -	**0** -	**0** -
Acacia pravissima	**0** 0	**0** +	**0** 0	**+** +	**+** +	**0** +	**+** +

not pollination limited, relative to Australia. This finding could be due to the introduction of widespread generalist pollinators to both ranges, most notably *Apis mellifera*, which pollinates *Acacia* in Australia and is known to pollinate introduced species in New Zealand (Bernhardt 1987; Moncur *et al.* 1991; Butz Huryn & Moller 1995; Goulson 2003; Howlett & Donovan 2010). Alternatively, or in addition, native pollinators present in New Zealand may perform functions similar to those in Australia. Most plant species in New Zealand are adapted for generalist pollination by insects, which may facilitate the pollination of introduced *Acacia* (Heine 1937; Norton 1984; Webb & Kelly 1993; Anderson 2003). The comparable levels of pod production and seed abortion in each country suggests that reproductive success in *Acacia* is robust to potential shifts in community assemblages associated with moving from one range to another, as expected from a generalist plant species.

In addition to being robust to shifts associated with introduction to new locations, the reproductive success of *Acacia* was also relatively consistent between sites within countries, since most variation in both pods per inflorescence and seed abortion rates occurred within individual trees. This is in contrast to studies in other systems that have found pollination success to be more spatially variable (Parker 1997; Ashman *et al.* 2004; Gómez *et al.* 2010), and may again be due to the now widespread introduction of generalist pollinators such as *Apis mellifera*. The only instance in which reproductive success varied greatly between sites was for *A. dealbata* in Australia (Appendix C), which showed complete reproductive failure at two sites and the highest reproductive success at another. Field observations suggested that the reproductive failure was due to the influence of natural enemies. However, that this was not significant in the analysis suggests that the method I used for scoring natural enemy attack may have been too coarse to accurately quantify differences, despite the presence or absence of natural enemies on branches being a good indicator of their influence elsewhere (Dennill & Donnelly 1991). Nevertheless, removing from the analysis the two sites where no pods were produced did not reveal a significant reduction in pollination success in New Zealand relative to Australia, suggesting that the overall conclusion would be the same.

The tendency for *A. pravissima* to produce heavier seeds in its native range in Australia may suggest that the quality of pollination is lower for this species in New Zealand, since self-fertilisation or variation in pollination intensity can result in the production of lower quality seeds in other genera (Wolfe 1995). However, that this was the only indicator of reproductive success that differed between the ranges may indicate that the difference in seed mass was due to other factors. Previous studies that have examined differences in seed mass between native and invasive species have found conflicting results. While some have found that species introduced to new locations can produce larger seeds than in their native range (Buckley *et al.* 2003; Daws *et al.* 2007), others indicate the opposite (Buckley *et al.* 2003; Mason *et al.* 2008). Although there is no consensus as to what factors may underpin differences in seed mass, latitudinal differences, a variable environment, or variation between individuals selected for planting and those growing under natural conditions have been implicated (McGinley *et al.* 1987; Aizen & Woodcock 1992; Buckley *et al.* 2003; Kitajima *et al.* 2006; Mason *et al.* 2008) and all could apply to this study. That the seed mass I recorded in New Zealand was the same as the mean seed mass recorded for this species elsewhere (Table 2.1) would suggest that the seeds produced by individuals in New Zealand are not unusual.

2.5.2 Predispersal seed predation

Although species introduced to new locations are predicted to escape their natural enemies, my data indicate that the extent to which enemy release occurs depends on both the species and the predator. Predispersal seed predation by birds was almost zero for all three species in New Zealand and considerably lower than in Australia. This likely reflects an overall lack of avian seed predators in New Zealand, relative to Australia. Seed feeders likely to be present at the study sites in New Zealand would be largely confined to introduced finches (Heather & Robertson 2000), while in Australia a range of finches, doves, pigeons and parrots will consume seeds (Whitney 2005; Twigg *et al.* 2009). However, as predispersal seed predation by birds was low in general, it is unlikely to have any considerable impact on reproductive output for the three species I examined.

In contrast, while predispersal seed predation by insects was also low for *A. pravissima*, particularly in New Zealand, *A. dealbata* and *A. baileyana* both experienced relatively high levels of predation in both countries and there was no evidence of escape from insect predispersal seed predators for these species. This is likely due to the long history of establishment of *Bruchophagus acaciae*, a wasp whose larvae develop in *Acacia* seeds, and that was introduced to New Zealand as early as the beginning of the 20th century (Hill

1999). While this is only one species, compared to several species that may attack *Acacia* in Australia, its impact may be greater in New Zealand due to the absence of its own natural enemies (Hill 1999). The finding that *A. dealbata* and *A. baileyana* experience similar levels of insect seed predation in the two countries is counter to expectations of the role of enemy release in facilitating invasion, particularly in the case of the highly invasive *A. dealbata*, but does support reports that *Acacia* accumulate natural enemies in their introduced range over time (Wingfield *et al.* 2011). However, since almost complete destruction of seeds must occur for predispersal seed predation to effectively impact *Acacia* populations (Impson & Moran 2004; Moran *et al.* 2004), the infestation rates I recorded are again unlikely to have severe impacts for species given the levels of seed production found in this and other studies (Gibson *et al.* 2011).

2.5.3 Overall reproductive output

Although examining the success of pollination and the influence of predispersal seed predation is important to identify variation in their strength between species and ranges, and thus their potential contribution to reproductive success, the overall reproductive output of plants is ultimately what matters for plant invasion. For *A. dealbata* and *A. baileyana* there were no differences in either the percentage of viable seeds per pod or overall seed rain and thus no suggestion that pollination or predispersal seed predation may differentially affect species in New Zealand relative to Australia. On a site by site basis there were rare instances where the combination of both seed abortion rates and predispersal seed predation led to an almost total seed loss for *A. dealbata* and *A. baileyana* (Appendix D), which highlights the potential for both pollination success and interactions with natural enemies to impact reproduction. However, this effect appeared to be highly localised and similar between the two countries.

In contrast, there was some indication that the performance of *A. pravissima* varied in New Zealand relative to Australia, and also relative to the other two species. While the increased percentage of viable seeds in *A. pravissima* compared to *A. dealbata* and *A. baileyana* seemed due to a combination of low seed abortion and low predispersal predation by insects, the cause of the high seed output of this species in New Zealand is less clear. Although not significant, there was a trend towards an increase in both the percentage of viable seeds and the absolute numbers of seeds in New Zealand which, combined with lower predation by birds, would likely lead to an increase in total seed output. However, these differences are unlikely to explain the magnitude of the increase in seed output I recorded. In addition, on a site by site basis, differences in fruiting efficiency (Appendix C) and viable seeds per pod (Appendix D) remained the same (and bird predation was consistently zero), while seed rain varied considerably (Appendix F).

Other research suggests that invasive species produce more seeds in their invaded than in their introduced range due to release from natural enemies allowing greater allocation to reproduction and/or canopy size, or due to selection of certain genotypes for introduction (Blossey & Nötzold 1995; Kitajima *et al.* 2006; Mason *et al.* 2008). An increase in total seed production has been demonstrated in other introduced *Acacia* (Noble 1989) and I found that *A. pravissima* trees in New Zealand were larger than those I examined in Australia (Appendix E). It is likely that a combination of the biotic interactions I examined together with either a more favourable environment or selection for certain genotypes underlie the variable and high seed output of *A. pravissima* in New Zealand. This finding highlights that despite being considered only as a casual species in New Zealand, and not recorded as invasive anywhere else in its introduced range, *A. pravissima* exhibits several attributes expected of a successful invader, which could support the suggestion by Gibson *et al.* (2011) that all Australian *Acacia* likely present high invasion risk.

2.5.4 Implications for plant invasion

Overall, my findings indicate that regardless of the mechanisms underlying the success of pollination or degree of enemy escape, neither pollination mutualisms nor predispersal seed predation appear to underlie the variable success of these three *Acacia* species in New Zealand. The highly invasive *A. dealbata* did not demonstrate any reproductive advantage in New Zealand relative to Australia, or relative to the other two species. Where I did find differences, they were in the opposite direction to what would be expected if interactions with pollinators and predispersal seed predators are important for invasive success, since *A. pravissima*, which is not invasive in New Zealand or anywhere else in the world, was at an advantage in terms of escape from seed predators. In addition, this species showed lower abortion rates in both ranges, suggesting it is less susceptible to pollination failure than the other two species.

Although reproductive success and output are generally considered important determinants of invasion success, this may only be the case where population growth is strongly seed limited. Since seed production in *Acacia* is high in absolute terms, and as seeds can persist in the seed bank for several decades (Brown *et al.* 2003; Richardson & Kluge 2008), seed limitation in *Acacia* is potentially less of a barrier to invasion than other factors. In particular, differences among species in dispersal ability and habitat requirements affecting recruitment success may be more important, or human-mediated influences such as propagule pressure or planting effort (Richardson, Williams & Hobbs 1994, Křivánek, Pyšek & Jarošík 2006, Dawson, Burslem & Hulme 2011, McGregor et al. 2012), and residence time (Křivánek, Pyšek & Jarošík 2006, Pyšek, Křivánek & Jarošík 2009) may confound any attempts to elucidate biotic drivers of invasion (Pyšek & Richardson 2007).

2.5.5 Limitations of the study

Acacia seed production can vary between years as species may alternate between light flowering years and heavy flowering years (Grant *et al.* 1994), pollination success may vary (Broadhurst & Young 2006) and levels of predispersal seed predation can also fluctuate (Hill 1999). The short duration (one season) of this study may therefore account for the low reproductive output I recorded, relative to that recorded for *Acacia* elsewhere (Gibson *et al.* 2011), and variation in reproductive success may not reflect differences in lifetime reproductive success of each *Acacia* species. However, a pilot study in New Zealand during the 2009-2010 reproductive season indicated that while overall seed output was higher in that year, the overall patterns between species were the same (Appendix G). In addition, given the overall success of *A. pravissima* relative to the other two species, it is unlikely that a longer period of study would highlight any significant advantage or disadvantage in terms of reproductive output that might explain their variable invasion success.

2.6 Conclusion

By examining the success of pollination and losses to predispersal seed predators of three species of *Acacia* in their native and introduced ranges I have shown that the reproductive success of *Acacia* is robust to introduction to new locations. However, examining species that have shown varying invasion success since introduction has demonstrated that, while reproductive traits may contribute to the success of *Acacia* as a group, they cannot explain the variable success of species within the group. The higher reproductive success and output of *A. pravissima* compared to two species that are more invasive in New Zealand may indicate the potential for this species to invade. finding underlines the need for greater research aimed at identifying factors currently limiting the invasive success of some *Acacia* species.

Chapter 3 - Mutualism vs. antagonism in introduced and native ranges: can seed dispersal and predation determine *Acacia* invasion success?

3.1 Abstract

Plant species introduced to new regions can escape their natural enemies but may also lose important mutualists. While mutualistic interactions are often considered too diffuse to limit plant invasion, few studies have quantified the strength of interactions in both the native and introduced ranges, and assessed whether any differences are linked to invasion outcomes. For three *Acacia* species adapted for ant dispersal (myrmecochory), we quantified seed removal probabilities associated with dispersal and predation in both the native (Australian) and introduced (New Zealand) ranges, predicting lower removal attributable to dispersal in New Zealand due to a relatively depauperate ant fauna. We used the role of the elaiosome to infer myrmecochory, and included treatments to measure vertebrate seed removal, since this may become an important determinant of seed fate in the face of reduced dispersal. We then tested whether differences in seed removal patterns could explain differences in the invasion success of the three *Acacia* species in New Zealand.

Overall seed removal by invertebrates was lower in New Zealand relative to Australia, but the difference in removal between seeds with an elaiosome compared to those without was similar in both countries. This implies that the probability of seed dispersal by invertebrates was comparable in New Zealand to Australia, but invertebrate granivory was higher in Australia. The probability of seed removal by vertebrates was similar and low in both countries. Differences in the invasive success of the three *Acacia* species in New Zealand were not explained by differences in levels of seed predation or the strength of myrmecochorous interactions. These findings suggest that interactions with ground foraging seed predators and dispersers are unlikely to limit the ability of *Acacia* species to spread in New Zealand, and could not explain their variable invasion success.

Key words: Biological invasion, density-dependence, establishment, exotic, plant-animal interactions, weed

3.2 Introduction

A leading hypothesis for the success of alien plant species introduced to new regions is that they benefit from leaving behind natural enemies, such as seed predators, that regulate the population in the native range (Keane & Crawley 2002). However, introduced plant species may also leave behind mutualists, such as seed dispersers, which are important in population spread. Although mutualistic interactions are often considered diffuse, in that many organisms can provide similar functions such that alien plants are likely to encounter suitable mutualists in novel environments (Horvitz & Beattie 1980; Pemberton 1988; Traveset & Richardson 2006), there is evidence that the loss of some mutualists can have negative impacts on alien plant performance that outweigh any advantages of enemy release (Morris *et al.* 2007; Pringle *et al.* 2009; Dickie *et al.* 2010). In addition, most studies that examine the role of mutualists in alien plant invasion focus on well-established and often problematic alien plants that are likely to have formed successful mutualisms (e.g. Glyphis *et al.* 1981; Rodríguez-Echeverría *et al.* 2003; Jensen & Six 2006). Consequently, the role that loss of mutualists might play in the failure of alien species to establish and spread may have been underestimated.

Seed dispersal is a key process in the establishment and spread of plant populations (Forget *et al.* 2005) and for most alien species sufficient seed must escape seed predators and be dispersed away from parent plants for successful invasion. While escaping natural enemies and forming new dispersal mutualisms may contribute to the success of some invasive species (Sakai *et al.* 2001; Buckley *et al.* 2006; Dawson *et al.* 2009), it is unclear whether failing to do so is the reason many other species fail to establish and spread. Understanding this requires quantifying biotic interactions in both the native and introduced ranges of alien plant species that differ in their invasive ability, comparisons that are rarely undertaken (Richardson *et al.* 2000a; Hierro *et al.* 2005). Here, we fill this gap by quantifying the importance of putative seed dispersers and seed predators in both the native and introduced ranges of three alien plant species that differ in the degree to which they have established and spread. We studied Australian species in the genus *Acacia* that have been introduced to New Zealand because here escape from a key dispersal mutualist could be critical in both preventing effective seed dispersal and increasing rates of seed predation.

Australian *Acacia* species have been introduced widely around the world, primarily for forestry and horticulture. While several species are invasive throughout their introduced range, many have failed to establish or spread following introduction (Richardson & Rejmánek 2011). Most Australian *Acacia* species have seeds adapted for dispersal by either birds (ornithochorous species) or ants (myrmecochorous species). Myrmecochorous species possess a lipid-rich appendage (elaiosome) that acts as a food reward and a handle to assist seed movement by ants (Mayer *et al.* 2005). Myrmecochory is thought to benefit seed dispersal through protecting seeds from predation and fire, by removing them underground, reducing intraspecific competition, by redistributing seeds away from parent trees, and increasing the rate of seed movement to more favourable microhabitats (Giladi 2006).

Myrmecochorous *Acacia* seeds fall to the ground soon after pod dehiscence. Once on the ground they are usually removed by ants, although birds, small mammals and other invertebrates may also remove seeds (Hughes & Westoby 1990). Predation of seeds by granivorous ants can account for almost all seed removal, depending on the species involved (Ireland & Andrew 1995). However, seed removal by ants can result in dispersal (myrmecochory) when ants transport seeds with an intact elaiosome to their nest, usually only a few metres from parent trees (Gómez & Espadaler 1998; Ness *et al.* 2004), and discard the seed once the elaiosome has been removed. Seeds are often discarded underground, where the hard coat of *Acacia* seeds allows them to persist in the seed bank for several decades (Brown *et al.* 2003; Richardson & Kluge 2008). Ants and other invertebrates may also eat the elaiosome *in situ* (Berg 1975; Auld 1986a; Beaumont *et al.* 2011), reducing the likelihood of subsequent ant dispersal (Auld 1986a; Ireland & Andrew 1995) and thus further leaving seeds exposed to predation (Hughes & Westoby 1990; Auld & Denham 1999).

At least 150 species of Australian *Acacia* have been introduced to New Zealand (Appendix A; Diez *et al.* 2009), of which 17 have naturalised, defined as having established self-sustaining populations (Howell & Sawyer 2006) and eight are sufficiently widespread to be classed as environmental weeds (Howell 2008). All but two of the species that have naturalised in New Zealand are myrmecochorous and the variable success of myrmecochorous *Acacia* species in establishing and spreading may be due to differences among species in their ability to form dispersal mutualisms, particularly as, relative to

Australia, New Zealand has a depauperate ant fauna with lower ant densities (Ward 2009). While there are more than 15,000 ant species in Australia (CSIRO 2012), and approximately 1500 myrmecochorous plant species (Berg 1975), New Zealand has only 11 native and 29 introduced ant species (Don 2007; Landcare Research 2012) and no confirmed native myrmecochorous plants (Thorsen *et al.* 2009). If seed dispersal by ants is important in *Acacia* population dynamics then these species may be at a disadvantage in New Zealand due to reduced seed dispersal, even if they concurrently escape ant granivory. Lower rates of removal by ants, and thus a decreased probability of burial, may also leave seeds exposed to vertebrate predators as introduced granivorous rodents are widespread in New Zealand (Beveridge 1964; Williams *et al.* 2000).

Since a function of the elaiosome is to elicit seed removal by ants (Berg 1975; Hanzawa *et al.* 1985; Auld 1986a; Aronne & Wilcock 1994; Pfeiffer *et al.* 2010), the increase in the removal rate of seeds with an elaiosome, relative to those without, is frequently used to infer the importance of myrmecochory in seed fate (Hughes & Westoby 1990; Pemberton & Irving 1990; Jensen & Six 2006). However, an increase in seed removal associated with elaiosome presence might not translate directly to dispersal if granivorous ants preferentially remove and consume those seeds (Hughes & Westoby 1990; Hughes & Westoby 1992a; Aronne & Wilcock 1994; Ireland & Andrew 1995). Determining the ultimate fate of seeds is difficult without following individual seeds, or excavating ant nests to estimate the proportion of seeds removed by ants that have been predated rather than discarded intact (e.g. Auld 1986a; Hughes & Westoby 1992b; Ireland & Andrew 1995). Nevertheless, when these studies have been undertaken, the results suggest that elaiosome presence does increase the probability of seed removal by ant species that disperse seeds (Auld 1986a; Ireland & Andrew 1995). In addition, these ants show a stronger preference for seeds with an elaiosome, relative to those without, than species that act mainly as seed predators (Hughes & Westoby 1992a; Hughes *et al.* 1994). Differences in the probability of removal for seeds with and without an elaiosome can therefore measure the strength of myrmecochory and thus the relative potential for seed dispersal.

We carried out diaspore removal experiments, where diaspore refers to the unit of dispersal (either the seed alone or the seed plus elaiosome), to quantify the probability of seed removal for three species of myrmecochorous *Acacia* that differ in the degree to which they have established and spread following introduction to New Zealand. We examined the probability of seed removal by invertebrates and used the presence or absence of an elaiosome to infer the strength of myrmecochory, and hence the relative potential for dispersal by ants, in both the native (Australia) and introduced (New Zealand) ranges. We also quantified the probability of removal by vertebrates, to identify whether vertebrate seed predation is more important where dispersal by ants is reduced.

We used this study system to answer three questions:

1. Does the probability of diaspore removal by invertebrates differ between New Zealand and Australia? We expect both higher overall removal probabilities and a stronger influence of the elaiosome on removal probability in Australia, relative to New Zealand, due to the more diverse and abundant ant fauna.
2. Does the probability of vertebrate removal differ between countries? In Australia seed removal by vertebrates is low, relative to invertebrates (e.g. Hughes & Westoby 1990), but this could differ in New Zealand if ant removal is reduced and because there is a different suite of vertebrate predators.
3. Can differences in the probability of seed removal by invertebrates and/or vertebrates explain the differential invasion success of *Acacia* species introduced to New Zealand? If seed dispersal and/or seed predation are important determinants of species' success we predict that more invasive *Acacia* species would have a higher probability of removal attributable to myrmecochory and/or lower seed predation probabilities.

3.3 Methods

3.3.1 Study species

From the pool of *Acacia* species that have been introduced to New Zealand we selected three myrmecochorous species that differed in the degree to which they have naturalised and spread (Table 3.1). All species are native to south-eastern Australia, a region with a close climate match to New Zealand (Kriticos 2012). *Acacia dealbata* Link is widespread and common throughout south-eastern Australia (Maslin 2001) and is invasive in New Zealand, where it has spread to form extensive monocultures along agricultural margins and in riverbeds. This species is also invasive in other parts of the world, particularly southern Africa, the Americas and Mediterranean Europe (Richardson & Rejmánek 2011).

Acacia baileyana F. Muell. is native to a small area around Cootamundra in New South Wales where it occurs in the forest understorey or in forest gaps (Maslin 2001), although it has been widely planted and is now naturalised beyond this range in Australia. In New Zealand, *A. baileyana* is a popular amenity tree and although widely naturalised it rarely spreads far from source populations, with only a few naturalised trees present at any locality. *Acacia baileyana* is considered invasive in Africa (Richardson & Rejmánek 2011). *Acacia pravissima* F. Muell. ex Benth is native to higher elevation zones of the Australian Great Dividing Range where it is found in open eucalypt forest and moist areas (Maslin 2001). In New Zealand it can reproduce successfully but has not spread beyond garden plantings and is currently classed as a casual (Howell & Sawyer 2006). There are no records of it being invasive elsewhere in the world (Richardson & Rejmánek 2011).

The three species differ in seed mass, elaiosome mass and elaiosome content (Table 3.1), which may influence the likelihood of removal by ants and vertebrates (O'Dowd & Gill 1986; Brew *et al.* 1989; Hughes & Westoby 1992a; Hughes *et al.* 1994; Pfeiffer *et al.* 2010).

3.3.2 Study sites and seed collection

In each country we selected up to five trees in up to five populations (sites) per species. The number of trees and sites we could sample was constrained by the logistics of undertaking all fieldwork during the period of seed release for each species in two countries (Table 3.1). We included multiple trees at each site to allow for variation in foraging behaviour that may result from between-tree variation in seed rain and/or canopy cover, and included multiple sites in each country to sample a range of environmental conditions. In Australia, populations were located within the species' known native ranges (Maslin 2001), but for logistical reasons we limited ourselves to searching for these within three hours drive of Canberra (35°16'S 149°7'E). We first located *A. baileyana* and *A. pravissima* populations and then selected populations of the more widespread *A. dealbata* close to these sites (see Appendix B). In New Zealand, study populations were located within a two hour drive of Christchurch (43°31'S 172°38'E). We included all *A. baileyana* and *A. pravissima* populations found with more than one individual (four of which contained fewer than five individuals), and five of the largest known *A. dealbata* populations (see Appendix B).

Table 3.1 Invasion status, phenology and seed characteristics for the three species of *Acacia* included in this study: *Acacia baileyana*, *Acacia dealbata*, *Acacia pravissima*. Seed characteristics include: mean dry seed mass, mean dry elaiosome mass, percent lipid content of the elaiosome and the percentage investment in the elaiosome (dry elaiosome mass/total dry diaspore mass).

species	**introduction date***	**invasion status**(1)(2)	**flowering**	**seed set**(3)(4)	**seed mass**(5)	**elaiosome mass**(5)	**% lipid**(5)	**% investment**(5)
A. dealbata	1868(6)	Invasive	July-Nov	Dec-Mar	9.1mg	0.4mg	46.1	4.0
A. baileyana	1916(6)	Naturalised	June-Aug	Oct-Jan	19.2mg	1.7mg	39.5	8.3
A. pravissima	1985(7) †	Casual	Aug-Nov	Jan-Mar	7.2mg	0.6mg	35.9	7.2
overall means for ant-dispersed *Acacia*(5):					**18.5mg**	**1.3 mg**	**36.5**	

* Date of earliest record found

† Earliest record but noted as widely planted as an ornamental at this time

(1) (Howell & Sawyer 2006)

(2) (Howell 2008)

(3) (Maslin 2001)

(4) (Gunn 2001)

(5) (O'Dowd & Gill 1986)

(6) (Papers Past New Zealand 2012)

(7) (Sheppard 1987)

Within each country we collected fresh, mature diaspores of each species from local populations and stored them at 4ºC to keep the elaiosomes fresh, diaspores were usually used within seven days of collection. At each site we ensured the diaspores used in the experiments were of the same species as the adult trees.

3.3.3 Experimental design

We used a removal experiment to estimate the probability diaspores were taken by either vertebrates or invertebrates in each country. To quantify the relative contribution of ants to diaspore removal we compared the removal of diaspores with and without elaiosomes and used this difference as an index of the strength of myrmecochory. We also recorded the number of diaspores that were not taken by invertebrates but that had their elaiosome removed.

Diaspores were presented in a Petri dish placed on the ground with an upturned lid secured on top and the entire unit fastened to the ground by a nail pushed through a central hole in the lower dish. Following Worthy *et al.* (2006) each unit was modified to enable selective access such that the lower dish allowed access to invertebrates only, while the upturned lid allowed access to vertebrates only. Invertebrate access to the lower dish was achieved by cutting small holes of ~5mm in the side of each dish and placing this flush with the ground with the diaspores inside and the upturned lid secured on top. To allow access by vertebrates only, diaspores were lightly glued to the upturned lid using an adhesive spray that was strong enough to prevent diaspore removal by any invertebrates that were able to gain access, but would not prevent diaspore removal by vertebrates. In addition to these selective access treatments, we included a no-access control treatment to measure background probabilities of diaspore loss. For this we enclosed the selective access dish in a wire cage with a mesh size of 5mm (to prevent vertebrate access) and placed it on a Perspex square that was covered in a thick layer of Tanglefoot® (to prevent invertebrate access).

To capture the range of factors that could influence vertebrate and invertebrate foraging behaviour, and therefore seed removal, we varied diaspore density and distance from a seed source (Hulme 1997). We presented either 1 diaspore (low density) or 20 diaspores (high density) at each location to account for the impact of density dependent foraging behaviour on seed survival. We also included two distance treatments: one at the base of each target tree and the second 10m from the canopy edge of the target and any other *Acacia* trees. In addition, since seed removal by ants in Australia can vary throughout the year often peaking in summer (Andersen & Ashton 1985; Hughes & Westoby 1990), and most *Acacia* seeds are removed within a relatively short period (Auld 1986a; Hughes & Westoby 1990; Ireland & Andrew 1995), we conducted the experiment in January and February to capture periods of both high ant activity and seed release for each species (Table 3.1). This also likely coincides with peak ant activity in New Zealand (Ward 2009) and ensured we were able to use fresh diaspores of each species.

Petri dishes were distributed to six locations around each tree: three at the base and three at 10m. Each location in a set of three was randomly allocated to one of three treatments: selective access dish with elaiosome intact, selective access dish with elaiosome removed or a no access control dish (which only ever contained diaspores with an intact elaiosome). At the beginning of the experiment each position at each tree was randomly assigned to one of the two density treatments. After four days the number of diaspores remaining was recorded and the dishes were given the second density treatment, using fresh diaspores for a further four days. This process was carried out once in January and once in February in each country. This resulted in a total of 48 observations per tree: 3 treatments comprising elaiosome removed, elaiosome intact and control x 2 access treatments (invertebrate and vertebrate) x 2 positions x 2 density treatments x 2 time periods (January and February). A total of 60 trees were sampled in each country (see Appendix B), resulting in 2880 observations of diaspore removal in Australia and New Zealand.

3.3.4 Statistical analysis

Rather than model diaspore removal as a two-stage process, examining encounter then removal, (e.g. Hulme 1994, 1996b; Baraibar *et al.* 2011), we estimated the overall probability of a diaspore being removed using a binomial distribution that included terms to model both variation among trees, which were nested within sites, and to account for overdispersion at high diaspore densities. The full details of the model are given in Appendix H.

We had six categorical treatment variables: diaspore type (elaiosome present or absent), *Acacia* species, country, distance, density and access type (invertebrate access only, vertebrate access only and no access control). Each treatment variable and associated interaction terms were included in statistical models by coding them as dummy variables and choosing one of the classes as a reference class with coefficient set to zero. We fitted two types of models to data subdivided by distance, density and access type. One model allowed for background loss of diaspores by including the no access control as a reference class, allowing us to visualise patterns in the data over and above background losses. The second excluded data from the no access control, providing a more direct test of the treatment differences we were interested in. Diaspore type, species and country were included as two or three level factor variables along with all interaction terms. Time was also included as a two level main effect factor (either January or February) to control for any difference in overall removal probabilities between the two time periods.

Models were fit using Markov chain Monte Carlo methods as implemented in OpenBugs (Thomas *et al.* 2006) called from the BRugs library in R v. 2.13.1 (R Development Core Team 2011). We ran three chains each with a burn in of 10000 iterations. The posterior distributions were then sampled from a further 10000 iterations of each chain, which were checked for convergence by visually inspecting the chain histories.

For each main effect or interaction term in the model, we tested its overall effect on diaspore removal by calculating the difference between the two classes associated with each term that had the most extreme coefficient values. We calculated this difference for each of the 30000 iterations (10000 from each chain) and then calculated the median and 95% credible intervals of these differences. We considered that a main effect or interaction term was significant if the 95% credible intervals of the differences did not overlap zero, implying a significant difference in removal probability between at least two of the classes associated with that term.

3.4 Results

Diaspore losses from the no access controls were low with diaspores having, on average, a 0.04 probability of being lost.

3.4.1 Invertebrate diaspore removal

There was a strong influence of the elaiosome on the probability of diaspore removal by invertebrates such that diaspores with an elaiosome were between 10 to nearly 1500 times more likely to be removed than those without (Figure 3.1). Removal probabilities were higher at high diaspore densities, and diaspores with an elaiosome were around 100 and 1500 times more likely to be removed than those without at the base of the tree and at 10m, respectively. At low diaspore densities, diaspores with an elaiosome were 10 times more likely to be removed than those without at both the base and 10m. These patterns were similar in both countries (no country related interactions in Figure 3.2).

Another consistent feature of invertebrate removal was a strong country effect in all models, showing that the overall probability of removal was higher in Australia than New Zealand regardless of whether an elaiosome was present or not (Figure 3.1e-h, country effects in Figure 3.2e-h). There was no significant country by diaspore type interaction, implying that the lower overall removal probabilities in New Zealand relative to Australia resulted from a reduction in removal of diaspores both with and without an elaiosome.

There was also an effect of *Acacia* species in all invertebrate models, with *A. dealbata* having lower diaspore removal probabilities than the other two species. At high diaspore densities this effect was further mediated by diaspore type (Figure 3.2f, h), suggesting that invertebrates differentially respond to density depending on the species and that the presence of an elaiosome had a weaker influence on removal probabilities for *A. dealbata*. *Acacia pravissima* showed more of an overall difference in removal probability between the two countries at the base of the tree and at high diaspore density (species x country interaction in Figure 3.2f).

When diaspores were presented with their elaiosomes, the few that remained at the end of the experimental period had often had their elaiosomes removed. Numbers were too low and variable for adequate analysis, but that this was frequently observed suggests that elaiosome removal has the potential to alter subsequent probabilities of diaspore removal.

3.4.2 Vertebrate predation

The overall probability of removal was lower for vertebrates than for invertebrates (0.16 compared to 0.44, respectively; Figure 3.1) in both countries. There was also less variation attributable to country, species and diaspore type on the probability of vertebrate removal than invertebrate removal (Figure 3.2). However, there were two sets of conditions under which vertebrate removal probability was lower in New Zealand than Australia, depending on the

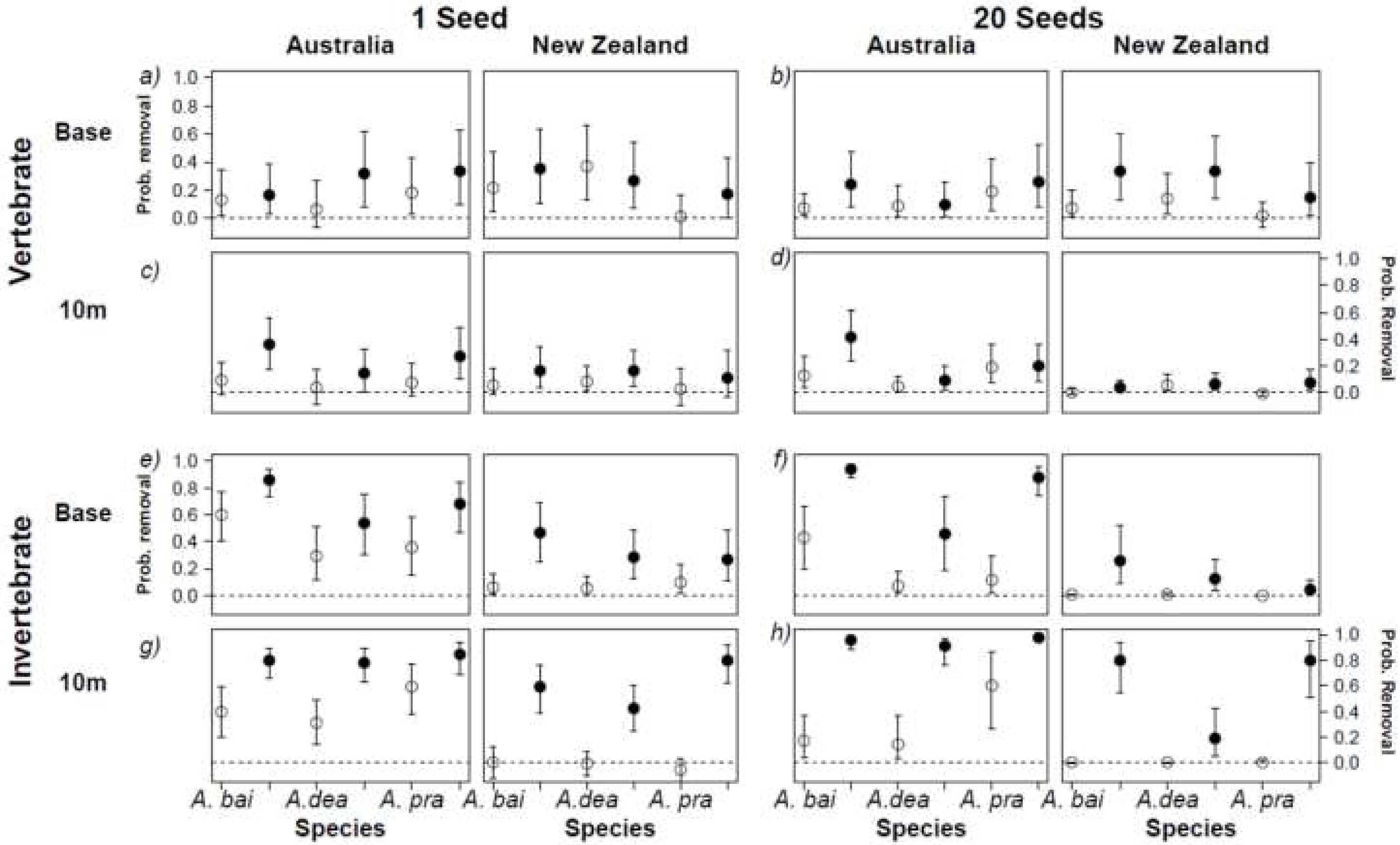

Figure 3.1 Posterior model probabilities of diaspore removal by vertebrates and invertebrates for three species of *Acacia* in Australia and New Zealand under varying seed densities and distance from the parent plant relative to the no access control (the reference class set to zero and indicated by the dashed line). Empty circles show the diaspore without an elaiosome treatment, filled circles show the diaspore with an elaiosome treatment, bars show the Bayesian 95% credible intervals. Treatments with credible intervals that do not overlap the dashed line at zero can be considered significantly different from the no access control. The data for each pair of graphs (a-h) were analysed separately.

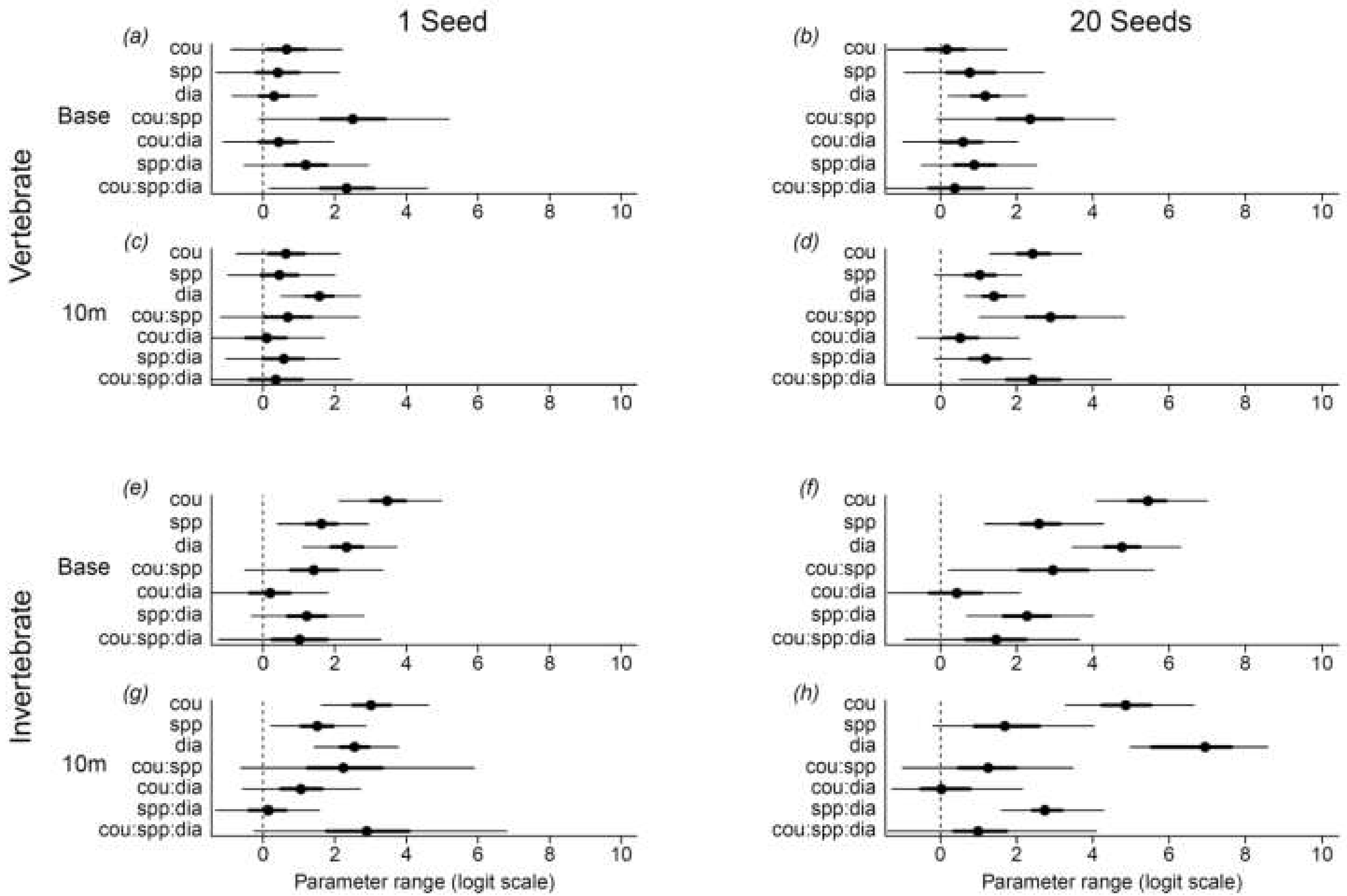

Figure 3.2 Parameter estimates (on the logit scale) for the effect of country (Australia, New Zealand), species (*A. baileyana*, *A. dealbata*, *A. pravissima*) and diaspore treatment (elaiosome removed, elaiosome present) and their interactions on the probability of diaspore removal by vertebrates and invertebrates, and how this varied by distance from the parent plant (base, 10m) or diaspore density (1, 20). Points show the mean of the posterior distribution for each parameter with 50% (thick lines) and 95% (narrow lines) credible intervals. cou: country; spp: species; dia: diaspore.

species involved (i.e. where there was a significant country × species × diaspore interaction): low diaspore density at the base of the tree (Figure 3.2a) and high diaspore density at 10m (Figure 3.2d). This was due to two species showing slightly different responses to the influence of elaiosome removal between countries (*A. pravissima* in Figure 3.1a and *A. pravissima* and *A. baileyana* in Figure 3.1d). In addition, the overall probability of diaspore removal was lower in New Zealand than Australia for diaspores at high density located away from parent plants (Figure 3.1d, Figure 3.2d).

The presence of an elaiosome also increased the probability of diaspore removal by vertebrates, although this effect was much less than for invertebrates: diaspores with an elaiosome were 3 to 5 times more likely to be taken by vertebrates than those without (Figure 3.2a-d). There was no evidence that this effect differed between countries (no significant country × diaspore interactions for vertebrates).

3.5 Discussion

Previous work suggests that interactions with seed dispersers are diffuse and introduced species are unlikely to be limited by leaving behind their natural dispersers as they are likely to encounter effective dispersers in the new range (Horvitz & Beattie 1980; Pemberton 1988; Richardson *et al.* 2000a; Stansbury 2001; Renne *et al.* 2002; Gosper *et al.* 2005; Traveset & Richardson 2006). Nevertheless, to our knowledge, this is the first study to directly quantify the probability of seed removal for introduced species in both their native and introduced ranges. By manipulating the elaiosome in diaspore removal experiments we were able to quantify the strength of myrmecochorous interactions for three *Acacia* species within each range and thus estimate potential seed dispersal and predation. We were, however, unable to determine the ultimate fate of diaspores after removal by ants, which will be largely contingent on the identity and behaviour of the ant species encountering the seeds (Berg 1975; Hughes & Westoby 1990; Hughes & Westoby 1992b; Bas *et al.* 2009). We found that the patterns of diaspore removal could not explain differences in invasion success for the three *Acacia* species in New Zealand.

3.5.1 Invertebrate diaspore removal in the introduced and native range

Overall diaspore removal by invertebrates was lower in the introduced (New Zealand) relative to the native (Australia) range. However, elaiosome presence had a similar effect on removal probability in both countries, suggesting that the strength of myrmecochorous interactions for *Acacia* are comparable in both Australia and New Zealand, despite a depauperate ant fauna and lower overall removal probabilities in New Zealand.

The high invertebrate removal probabilities we recorded in Australia support other studies that find rapid removal of *Acacia* seeds during periods of seedfall in their native range (Auld 1986a; Hughes & Westoby 1990; Ireland & Andrew 1995). That overall removal was higher than in New Zealand likely reflects the greater diversity (Don 2007; CSIRO 2012; Landcare Research 2012) and abundance (Ward 2009) of ants present in Australia. Our finding that this was due to increased removal probabilities of diaspores both with and without an elaiosome would suggest much higher probabilities of removal by granivorous ants in Australia than New Zealand, since elaiosome presence/absence has less influence on ant species that function more as seed predators (Hughes & Westoby 1992a; Hughes *et al.* 1994). It may also highlight that seeds are more of a limiting resource in Australia, as ants may more readily remove seeds without an elaiosome when there is no alternative food source (Ireland & Andrew 1995). That we found high rates of elaiosome removal from diaspores that remained in dishes at the end of each experimental period indicates that diaspores not removed by seed harvesting ants will have significantly reduced chances of dispersal in Australia.

The removal patterns we found for invertebrates in New Zealand (lower overall diaspore removal but a similar increase in removal associated with elaiosome presence as Australia) suggest the elaiosome rather than the seed is consistently the reward. This could arise if the probability of removal by truly granivorous ants was lower in New Zealand but potentially beneficial myrmecochorous interactions occur to the same degree as in Australia. Two possible and non-exclusive explanations for the patterns of diaspore removal we recorded are evident from the ant species known from the New Zealand study area (Table 3.2). First, diaspore removal could be carried out by generalist or carnivorous species. One of the reasons myrmecochory is considered diffuse is that the elaiosome can elicit removal by a range of ant species, not necessarily seed specialists (Brew *et al.* 1989; Hughes *et al.* 1994). If so, such species would be unlikely to remove diaspores without an elaiosome. Second, two native seed harvesters as well as two introduced species of Australian *Pheidole* have been recorded in the area and could be responsible for seed removal. *Pheidole* species act as both predators and dispersers of *Acacia* in their native range (Auld 1986a; Hughes & Westoby 1990; Hughes & Westoby 1992a,b; Ireland & Andrew 1995;

Beaumont *et al.* 2011) and the low levels of granivory implied by our results could reflect a lower abundance of these ants in New Zealand relative to Australia (Ward 2009). In addition, these species might also be more selective when seeds are not limiting and remove only diaspores with an elaiosome. If seed harvesters are responsible for diaspore removal, a proportion of the diaspores removed may undergo subsequent predation. As in Australia, the removal of elaiosomes from diaspores *in situ* indicates that those diaspores not removed initially will have little opportunity for later dispersal by ants.

Ant species present in New Zealand, both native and introduced, are small relative to the global mean of ant species involved in myrmecochory (Table 3.2). This could influence the quality of seed dispersal by reducing the distance seeds are dispersed (Auld 1986a; Ness *et al.* 2004), or increasing the likelihood they are discarded aboveground and therefore not protected from predation (Auld 1986a). Nevertheless, most ant species in New Zealand fall within the size range of those recorded dispersing *Acacia* seeds in Australia (Ness *et al.* 2004), suggesting effective dispersal should still occur. In addition, since ant granivory in New Zealand appears less common than in Australia, and the probability of vertebrate removal is low, the cost of being discarded aboveground may be lower. Thus, although we cannot identify the ant species responsible for seed removal, and so determine the ultimate fate of seeds, our findings indicate that even a low diversity of ant species in New Zealand, potentially both native and introduced, enable species to establish dispersal mutualisms. This suggests that myrmecochorous *Acacia* species are unlikely to suffer from a lack of dispersal mutualists when introduced to new locations given that New Zealand has a very depauperate ant fauna and other depauperate locations, for example oceanic islands, frequently have widespread introduced ant species (Wilson & Taylor 1967; Morrison 1997).

3.5.2 Vertebrate diaspore removal in the introduced and native range

The overall probability of vertebrate seed removal was low relative to invertebrate removal in both Australia and New Zealand and highlights the key role that invertebrates play in the fate of *Acacia* seeds in Australia. Apart from lower invertebrate granivory, the overall patterns of seed removal were thus very similar between New Zealand and Australia, despite potentially little overlap in the species responsible. We observed higher removal by vertebrates at low diaspore densities, suggesting that *Acacia* species do not escape seed predators by being at a low density. This also suggests that the lower probabilities of seed

Table 3.2 Ant species known from the study area in New Zealand (Canterbury), their status, size and diet (Don 2007; Landcare Research 2012). C: carnivorous; G: generalist; S: seed harvester. Species introduced from Australia are indicated.

species	status	length (mm)	diet	species/genus recorded dispersing *Acacia*?
Amblyopone saundersi	Endemic	3.0 - 5.5	C	
Doleromyrma darwiniana	Introduced (Australia)	2.0	G	
Linepithema humile	Introduced	2.2 - 2.6	G	
Technomyrmex jocosus	Introduced (Australia)	3.0 - 3.4	G	
Nylanderia spp	Introduced (possibly Australia)	3.0	G	
Prolasius advenus	Endemic	2.9 - 3.5	G	
Huberia brounii	Endemic	3.2 - 3.5	G	
Huberia striata	Endemic	4.5 - 5.0	G	
Monomorium antarcticum	Endemic	3.0 - 5.0	G/S	Yes (1)†
Monomorium pharaonis	Introduced	1.5 - 2.4	G	Yes (1)†
Monomorium smithii	Endemic	2.3-2.6	G/S	Yes (1)†
*Pheidole megacephala**	Introduced (probably via Australia)	3.5	G/S	Yes (2) (3) (4)
Pheidole rugosula	Introduced (Australia)	3.0	G/S	Yes (2) (3) (4)
Hypoponera eduardi	Introduced	2.6 - 3.0	C	
Discothyrea antarctica	Endemic	2.0	C	
	mean (using mid-point) ‡	**3.0**		
	range ‡	**1.5 - 5**		

* (O'Donnell 2008)

† Members of genus also noted as frequent elaiosome thieves (Beaumont *et al.* 2011)

‡ Mean size of ants recorded in myrmecochory globally is 5.2mm in the range 2 - 10mm; mean seed size of seeds involved is 19.34 in the range 11.5 – 40mm (Ness *et al.* 2004)

(1) (Ness *et al.* 2004 and references therein)

(2) (Auld 1986b)

(3) (Hughes *et al.* 1994)

(4) (Ireland & Andrew 1995)

removal by invertebrates in New Zealand are unlikely to subsequently expose diaspores to higher rates of vertebrate diaspore removal. Although we can only infer the identity of foraging vertebrates, in New Zealand seed predators will be introduced granivorous rodents, particularly *Mus musculus* L. and *Rattus rattus* L. (Moles & Drake 1999; Wilson *et al.* 2007; Wilson & Lee 2010), which are widespread in both native and human-modified environments. In Australia these species would be present too, although native vertebrates, such as *Rattus rattus* and *Wallabia bicolor*, may also be foraging in the semi-natural ecosystems we investigated (Auld & Denham 1999).

In contrast to previous studies (Auld & Denham 1999), vertebrates showed a preference for diaspores with an elaiosome in both ranges, although to a much lesser extent than shown by invertebrates. This could arise because small mammals respond to olfactory cues and may more easily locate diaspores with an elaiosome (Vander Wall 1998). If so, it highlights a trade-off these species face between making their seeds attractive to ant dispersers and increasing the probability of vertebrate predation (Buckley *et al.* 2006). However, the influence of the elaiosome on vertebrate removal was small relative to its influence on invertebrate removal, suggesting the costs are low compared to the benefits.

3.5.3 The role of seed fate in the invasion success of *Acacia* in New Zealand

There was little variation in patterns of seed removal between species and any variation we did observe did not explain the variable invasion success of the three *Acacia* species. In particular, the invasive *A. dealbata* showed no evidence of being at an advantage in terms of either lower levels of predation or increased dispersal. It is possible that the larger population sizes of this species in New Zealand, relative to the other *Acacia* species, could have masked differences in removal probabilities for vertebrate and/or invertebrate removal. Specifically, the high population size might increase the probability of vertebrate predation, as vertebrate foraging behaviour has been shown elsewhere to exhibit positive density-dependence (Hulme & Borelli 1999). However, in our experiment vertebrate removal probabilities were lower in the high density treatment. In addition, since vertebrate predation probabilities were low, it is unlikely that any decrease in predation associated with small initial population sizes would significantly alter invasion success. For invertebrate removal, the high population size might mask ant preference for this species due to ant satiation at high diaspore densities. However, when we presented diaspores of each species underneath trees of all three *Acacia* species in New Zealand, there were no strong patterns of invertebrate preference despite generally lower removal from under *A. dealbata* (see Appendix I). It is thus unlikely that, had we examined *A. dealbata* at an earlier stage of invasion, we would have found patterns of diaspore removal that could explain its greater invasion success.

Our finding that diaspores of the three *Acacia* species had similar removal probabilities suggests that their diaspores are not sufficiently different to affect their likelihood of dispersal or predation in New Zealand. One explanation for this is that the seed size of all three species falls within the range that allows collection by the ant species present in New Zealand, since size is posited to be the most important influence on seed removal (Beattie *et al.* 1979; Hughes & Westoby 1992a). Together with low vertebrate predation, our findings indicate that, irrespective of diaspore removal dynamics in the native versus the introduced range, animal-mediated seed predation and dispersal do not determine the relative success of the study species in New Zealand.

Seed fate may not be strongly tied to population performance where species can reproduce vegetatively, where regeneration is microsite rather than seed limited and/or where seed losses to predators are buffered by a large and persistent seed bank (Hulme 1996a). Since some species of *Acacia*, notably *A. dealbata*, are able to reproduce through sprouting (Maslin 2001), and *Acacia* seeds can persist in the seed bank for several decades (Brown *et al.* 2003; Richardson & Kluge 2008), the variable success of *Acacia* in New Zealand could be due to differences among species in their habitat requirements and/or interactions with enemies and mutualists at other stages of establishment, combined with human-mediated influences such as propagule pressure or planting effort (Richardson *et al.* 1994; Křivánek *et al.* 2006; Dawson *et al.* 2011; McGregor *et al.* 2012), and residence time (Křivánek *et al.* 2006; Pyšek *et al.* 2009b). As the relative distribution and status of the three *Acacia* species matches their known introduction date, with the widespread *A. dealbata* having been introduced almost a century before the casual *A. pravissima*, the patterns observed may simply reflect the long lag-phases that are commonly seen between a species naturalising and subsequently spreading in New Zealand (Aikio *et al.* 2010) rather than fundamental life-history or dispersal attributes (Wilson *et al.* 2007).

In this study we have shown that both dispersal and predation of *Acacia* seeds involve diffuse interactions, with similar outcomes in two environments independent of the suite of interacting organisms. By studying introduced plant species in their native and introduced ranges, and including species with differing invasion success, we have been able to demonstrate that, while the balance between dispersal and predation experienced by these three *Acacia* species in New Zealand is sufficient to allow establishment, it does not explain differences in their subsequent ability to spread.

Chapter 4 - Rhizobial limitation of spread in alien plants: a reduction in rhizobial availability limits the performance of introduced *Acacia* in New Zealand but cannot explain invasion outcomes.

4.1 Abstract

1. The ability to form effective mutualisms with nitrogen-fixing bacteria (rhizobia) is implicated in the success of introduced leguminous plant species, such as *Acacia*. While *Acacia* appear to form symbiotic associations with rhizobia where introduced, there is evidence that a failure to do so may limit success during early stages of colonisation.
2. We examine the growth of three Australian *Acacia* species that have been introduced to New Zealand in soils collected from both the native and introduced range, and ask whether variation in their ability to form rhizobial associations can explain differences in the invasive success of these species.
3. In a glasshouse experiment we measured growth and nodulation of *Acacia* seedlings grown under four soil treatments: soils taken from underneath conspecifics (Host+ soils) in both Australia and New Zealand, and soils taken from the same sites but away from *Acacia* trees (Host-). We predicted that growth and nodulation in the native range (Australia) would be similar in Host+ and Host- soils due to the widespread presence of *Acacia*. In New Zealand, however, we predicted that growth and nodulation would be lower in Host- relative to Host+ soils, due to low availability of suitable rhizobia away from established conspecifics. We also predicted that such limitation would be lower in more invasive species of *Acacia*. In addition, we examined whether seedling growth rates and nodulation differed in soil taken from beneath congeners, relative to conspecifics, to determine whether the establishment of one species of *Acacia* might facilitate establishment of other species.
4. As predicted, seedling growth and nodulation were lower in Host- soils relative to Host+ soils of congeners in New Zealand, but there was no significant difference in Australia. There was no significant difference in seedling growth rate between soils collected under congeners relative to soils collected under conspecifics.
5. Our results show that in New Zealand *Acacia* seedlings colonising sites away from established conspecifics or congeners will suffer reduced growth and nodulation,

which may limit the ability of *Acacia* species to spread, relative to in the native range. Interactions with rhizobia, and soil biota in general, thus have the potential to limit the establishment of species colonising new sites in the introduced range. The degree of limitation, however, was similar for all three species, implying that interactions with soil biota cannot explain the variable invasive success of these *Acacia* in New Zealand.

4.2 Introduction

Some plant species introduced to new regions appear to leave behind soil pathogens that regulate populations in the native range, which may give them an advantage over other species in establishment and spread (Klironomos 2002; Reinhart *et al.* 2003; Reinhart & Callaway 2006). However, for species that rely on symbioses with soil biota, such as leguminous species that are dependent on nitrogen-fixing bacteria (rhizobia) for establishment into many soils, leaving behind their mutualistic soil biota may be a disadvantage unless suitable mutualists are also present in the introduced range (Burdon *et al.* 1999; Parker *et al.* 2006). Although studies have found that many invasive plant species have successfully formed mutualistic associations with rhizobia in introduced ranges (Rodríguez-Echeverría *et al.* 2003; Parker *et al.* 2007; Callaway *et al.* 2011; Porter *et al.* 2011; Rodríguez-Echeverría *et al.* 2011), there is evidence that rhizobia may be more limiting when species first colonise new sites (Parker *et al.* 2006; Stanton-Geddes & Anderson 2011), which could limit their ability to spread in novel environments. If so, variation in the extent to which species' experience such limitation may help explain the variable invasive success shown by exotic legume species in different locations.

Although rhizobia are present in many soils, the ability of plant species to form viable symbioses (effective nodules) is dependent on both the identity and density of bacteria available: highly promiscuous plant hosts are able to nodulate with a wide range of strains and at low bacterial densities, while less promiscuous hosts show greater strain specificity and require higher bacterial densities before they nodulate (Roughley 1987; Bhuvaneswari *et al.* 1988; Thrall *et al.* 2000; Thrall *et al.* 2005; Thrall *et al.* 2007). In addition, plant hosts themselves influence the availability of rhizobia in the soil (Thrall *et al.* 2000) as rhizobia population numbers increase rapidly in response to compatible plant hosts (Purchase & Nutman 1957; Parker 2001) and can decline quickly when they are absent (Thrall *et al.* 2001).

The Australian *Acacia* are a diverse group of leguminous trees and shrubs that are widely cultivated outside their native range, primarily for forestry and horticulture. A relatively high proportion (around 6%) of species introduced to new regions has succeeded in establishing outside of cultivation and spreading into native ecosystems (Richardson *et al.* 2011). This is partly due to their ability to establish into nutrient poor soils, which is facilitated by interactions with rhizobia, and several studies have focused on this genus as a model system with which to study the role of rhizobia in determining invasion outcomes (Rodríguez-Echeverría *et al.* 2009; Rodríguez-Echeverría *et al.* 2011; Birnbaum *et al.* 2012).

In species' native ranges rhizobia with which *Acacia* can nodulate appear to be widespread (Barnet & Catt 1991), which may be because congeneric species can often share rhizobia and *Acacia* are a dominant component of many habitats (Thrall *et al.* 2000; Birnbaum *et al.* 2012). Current evidence also suggests compatible rhizobia are present in much of the introduced range of *Acacia*, with species recorded nodulating in Europe (Rodríguez-Echeverría *et al.* 2009), Asia (Midgley & Vivekanandan 1987; Le Roux *et al.* 2009; Ma *et al.* 2012), Africa (Mohamed *et al.* 2000; Joubert 2002; Rodríguez-Echeverría 2010; Boukhatem *et al.* 2012) and the Americas (Aronson *et al.* 1992), as well as outside their native range in Australia (Birnbaum *et al.* 2012). Although it is not clear what facilitates species' nodulation outside their native range, the widespread occurrence of cosmopolitan rhizobia (Weir *et al.* 2004; Birnbaum *et al.* 2012) or the co-introduction of compatible rhizobia from their native range have both been implicated (Rodríguez-Echeverría 2010; Birnbaum *et al.* 2012; Ndlovu *et al.* 2013).

However, despite the apparent ubiquity of suitable rhizobia, there are many more species that have failed to spread away from introduction sites than have succeeded. A potential reason for this is that although suitable rhizobia are geographically widespread, their availability in the soil is limiting. Low densities and a patchy distribution of compatible rhizobia has been suggested to limit the establishment of other invasive legumes (Parker *et al.* 2006) and there are examples of *Acacia* failing to nodulate or showing reduced performance in some introduced soils (Turk *et al.* 1993; Weir 2006). Since previous studies that examine interactions between alien *Acacia* and rhizobia focus on the performance of *Acacia* species that are already known to be invasive in soils taken from underneath established individuals that we would expect to have successfully encountered suitable rhizobia (e.g. Mohamed *et al.* 2000; Rodríguez-Echeverría 2010; Birnbaum *et al.* 2012), the availability of suitable rhizobia for all *Acacia* species introduced to new locations may have been overestimated.

Here, we aim to examine the extent to which rhizobial availability in the introduced range of *Acacia* might influence species' ability to spread away from introduction sites and thus determine invasion outcomes. We do this by determining whether the availability of rhizobia might limit plant performance away from established individuals under natural conditions in the introduced range when compared to species' native ranges, and do this across species that vary in invasion success. In addition, since many *Acacia* species can share rhizobia, we also determine whether the presence of an established congener might influence the availability of rhizobia and facilitate the establishment of other *Acacia* species. This is the first examination of plant performance in soils from both beneath established *Acacia* and away from established individuals in both the native and introduced ranges, and so the first direct test of limitation for seedlings colonising new locations, as well as the first study to examine interactions between rhizobia and species that vary in invasive success.

New Zealand has no native *Acacia* species but at least 150 species of Australian *Acacia* have been introduced (Appendix A; Diez *et al.* 2009). Although the majority have remained close to introduction sites, eight species have spread and are considered environmental weeds (Howell 2008). Of those that have spread, all except one are considered invasive in other parts of their global introduced range (Richardson & Rejmánek 2011). There is little information on the interaction between *Acacia* and rhizobia in New Zealand. Although nodules were collected from an established population of *A. longifolia*, a species that is invasive in some parts of New Zealand, the same species did not nodulate in soils collected away from established individuals (Weir 2006). This indicates that rhizobia may be limiting for species at early stages of colonisation in New Zealand, which is further supported by the finding that although cosmopolitan rhizobia capable of nodulating *Acacia* species are present in New Zealand, they do not appear to associate with native species and are therefore likely at low densities (Weir *et al.* 2004; Weir 2006).

By selecting three *Acacia* species that vary in the degree to which they have spread following introduction to New Zealand we tested the following hypotheses:

1. That plant performance and nodulation is reduced away from established populations of *Acacia* in the introduced (New Zealand) range due to low rhizobial availability but not the native (Australian) range due to the widespread presence of compatible rhizobia.
2. That there is species-specific variation in the extent to which performance is reduced away from conspecifics in New Zealand and that this correlates with how widespread the species is in New Zealand.
3. That rhizobial populations associated with naturalised congeners can improve plant performance and could therefore facilitate the establishment of *Acacia* arriving in new locations.

4.3 Methods

4.3.1 Study species

The three species we selected have all successfully naturalised in New Zealand, indicating that compatible rhizobia are present to some extent, but differ in the degree to which they have spread away from introduction sites (Table 4.1). The species are all native to south-eastern Australia, a region with a close climate match with New Zealand (Kriticos 2012). *Acacia dealbata* Link is widespread and common throughout south eastern Australia (Maslin 2001) and is highly invasive in New Zealand, where it forms extensive monocultures along agricultural margins and in riverbeds. It is also considered invasive in other parts of the world, particularly southern Africa, the Americas and Mediterranean Europe (Richardson & Rejmánek 2011), and has been recorded nodulating throughout its introduced range, including South Africa (Joubert 2002), Chile (Aronson *et al.* 1992), Sri Lanka (Midgley & Vivekanandan 1987) and China (Ma *et al.* 2012). The native range of *A. dealbata* overlaps that of both subsequent species we selected. *Acacia baileyana* F. Muell. is native to a small area around Cootamundra in New South Wales where it occurs in the forest understorey or in forest gaps (Maslin 2001), although it has been widely planted and is now naturalised beyond this range in Australia. In New Zealand, *A. baileyana* is a popular cultivated tree and although widely naturalised rarely spreads far from source populations. *Acacia baileyana* is considered invasive in Africa (Richardson &

Rejmánek 2011). Although it may nodulate with a variety of strains within Australia (Roughley 1987), its association with rhizobia does not appear to have been studied outside its native range. *Acacia pravissima* F. Muell. ex Benth is native to higher elevation zones of the Australian Great Dividing Range where it is found in open eucalypt forest and moist areas (Maslin 2001). In New Zealand it can reproduce successfully close to parent trees but has not spread beyond garden plantings and is currently classed as a casual (Howell & Sawyer 2006). There are no records of it being invasive elsewhere in the world (Richardson & Rejmánek 2011).

Table 4.1 Invasion status and introduction date for the three species of *Acacia* included in this study: *Acacia baileyana*, *Acacia dealbata*, *Acacia pravissima*.

Species	**Introduction date***	**Invasion status**$^{(1)(2)}$
A. dealbata	1868$^{(3)}$	Invasive
A. baileyana	1916$^{(3)}$	Naturalised
A. pravissima	1985$^{(4)\dagger}$	Casual

* Date of earliest record found
† Earliest record but noted as widely planted as an ornamental at this time
(1) (Howell & Sawyer 2006)
(2) (Howell 2008)
(3) (Papers Past New Zealand 2012)
(4) (Sheppard 1987)

4.3.2 Experimental design

To quantify the extent to which seedling performance is limited by rhizobial availability in New Zealand, relative to Australia, we conducted a glasshouse experiment using field soils. We measured seedling growth and examined the extent to which patterns of nodulation could explain variation in plant performance. Using whole soils such as this has the advantage of allowing us to examine the net effect of the soil biota associated with conspecific or congeneric trees on plant performance. We can then identify the potential contribution of rhizobia to this by correlating growth with the numbers of nodules produced. A disadvantage of using whole soils is that we are unable to explain any variation in plant performance that cannot be attributed to rhizobia.

In each country we collected two types of soils: from underneath established conspecifics (Host+) and from the same sites but 20 m away from conspecifics and any other *Acacia*

(Host-). We considered this distance to be sufficient to escape any effects of *Acacia* trees and their root systems on soil communities (see also Callaway *et al.* 2011) but close enough to ensure other soil properties were similar and therefore control for variation in soil properties that are not attributable to the presence of an *Acacia* host. Because populations of rhizobia increase in the presence of their host plants, the Host+ soils are representative of plant performance when rhizobia are not limiting, while the Host- soils represent the soil conditions experienced by individuals spreading away from source populations. We used the difference between plant performance in the Host+ and Host- soils as a relative measure of the extent of rhizobia limitation. To examine whether the presence of naturalised congeners might facilitate plant establishment we also carried out a cross-inoculation experiment and planted seedlings of each species into the Host+ and Host- soils of the other two congeneric study species in Australia and New Zealand.

4.3.3 Study sites and soil collection

Because nodulation with rhizobia can be influenced by soil properties and environmental conditions (Vincent 1965; Habish & Khairi 1970), we collected soils from multiple populations (sites) in Australia and New Zealand to ensure we captured a range of soil conditions. We identified four sites per species in Australia and five sites per species in New Zealand (Appendix B). In Australia populations were located within the species' known geographic range (Maslin 2001) but for logistical reasons we limited ourselves to searching for these within a three hour drive of Canberra (35º16'S 149º7'E). We located four populations each of *A. baileyana* and *A. pravissima* that contained five or more adult individuals, and these were included as study sites. *Acacia dealbata* was more widespread around Canberra and, for logistical ease, we chose four populations located close to the *A. baileyana* and *A. pravissima* populations (see Appendix B). The three species did not co-occur at any of our study sites. In New Zealand, study populations were located within a two hour drive of Christchurch (43º31'S 172º38'E). We included all *A. baileyana* and *A. pravissima* populations with more than one individual we could find, five of which contained fewer than five individuals, and included the five largest *A. dealbata* populations (Appendix B). In New Zealand *A. baileyana* and *A. pravissima* co-occurred at two sites due to the two species often being planted together as ornamentals (Appendix B). In this situation we ensured that soils were sampled from individuals that were at least 20 m away from each other.

For the Host+ soil treatment we collected soil from underneath the base of up to five haphazardly selected trees of each species at each site. Soils were collected to a depth of 10cm, excluding the litter layer, and pooled together to form one Host+ soil sample per site. For the Host- soil treatment we took several samples per site, again to a depth of around 10cm, and pooled these together to also form one Host- soil sample per site. We did not make an effort to avoid other legume species when collecting Host- soils because a key aim of this study was to examine the growth of *Acacia* seedlings in those soils they would actually encounter when spreading away from parent plants. In Australia we collected a total of 24 soil samples: 2 host treatment soils (Host+ and Host-) × 3 host species (*A. baileyana*, *A. dealbata*, *A. pravissima*) × 4 sites per species. In New Zealand we collected a total of 30 soil samples.

After collection soils were stored in large paper bags for transfer to the glasshouse where they were air dried for up to 48 hours. Soils were then sieved to remove any stones and other dry matter and stored in paper bags at room temperature until use (<2 weeks).

4.3.4 Glasshouse experiment

Glasshouse experiments were conducted separately in Australia and New Zealand. Conducting the experiments separately means that the results cannot be directly compared between countries, due to potential differences in glasshouse conditions. For this reason, when testing for between-country differences we use the relative difference (effect size) between Host+ and Host- soils as a measure of plant performance, rather than absolute variation.

We obtained seed from the Australian Seed Company and used seeds from the same seed lot in both the native and the introduced range to control for any differences that may arise from fitness variation in seeds of different provenances. Seeds were germinated by boiling them in water for 1 minute then leaving them to imbibe overnight, following the Australian Seed Centre Manual (Gunn 2001). They were then transferred to germination trays containing a 1:1 mixture of sterile vermiculite: sand and watered as required until germination.

When seedlings reached the first leaf stage we transplanted them into each of the treatment soils. To do this, we filled pots (150mm height, 80mm diameter) to ¾ with 1:1 sterilized vermiculite: sand mixture. We then covered each pot with 100ml of one of the soil treatments to serve as an inoculant for the seedlings and covered this with a further 1cm layer of sterile soil. This protocol follows Thrall *et al* (2007) and the relatively small amount of soil used is intended to further control for any effects of soil chemistry and nutrient status on plant growth. The soils of each pot were covered with polyurethane beads (approximately 2 mm in diameter) to prevent cross contamination during watering. We also included sterile controls for each species where seedlings were planted into pots filled only with the sterile vermiculite: sand mixture and covered with polyurethane balls.

Pots were placed in a randomized block design. Each block consisted of one seedling of each species planted into each of the soil treatment types, as well as two sterile controls per species. In Australia this resulted in 72 seedlings/pots per block: 24 soil samples (as described above) × 3 seedlings species (*A. baileyana*, *A. dealbata*, *A. pravissima*), as well as the 6 additional seedlings in the sterile controls (78 seedlings in total). This was repeated six times (6 replications of each combination of seedling species and soil treatment type), which gave an overall total of 468 seedlings in Australia. In New Zealand this resulted in a total of 90 seedlings/pots per block: 30 soil samples × 3 seedling species, as well as the additional 6 control seedlings. Here, 7 replicates were used, giving an overall total of 672 seedlings in New Zealand. The position of each pot within each block was randomly assigned.

Seedlings were grown under an 18-24°C temperature regime with ambient light conditions. In Australia there were several days when temperatures exceeded this due to a combination of particularly hot weather and problems with the air conditioning system. Seedlings were grown for 14 to 16 weeks in Australia and 16 to 18 weeks in New Zealand. The difference in timing was due only to time constraints and seedlings were large enough at 14 weeks for any differences to be observed. If seedlings died within the first few days they were replaced. As seedling mortality was low, seedlings that died after the first few days were removed from the analysis. Seedlings were watered with N-free 1:20 diluted McKnight's solution (McKnight 1949) three times a week and tap water if needed otherwise. Pots were weeded regularly to ensure that seedling growth was not affected by competition with other plants.

We used plant growth rate as our measure of plant performance across treatments. To calculate this we harvested the aboveground parts of the plants and oven dried them at 70°C for 48 hours before weighing. The growth rate of each plant was then calculated as aboveground plant dry weight / the number of days since planting (g day^{-1}). To assess the importance of interactions with rhizobia for plant growth and identify whether patterns of nodulation were linked to plant performance we used the total number of effective nodules as a measure of symbiotic success. Nodule number has been found as a good indicator elsewhere (Thrall *et al.* 2007). Effective nodules can be scored as to their size, colour and position, and we considered any nodule to be effective if it was pink to red in colour (Corbin *et al.* 1977; Thrall *et al.* 2007), indicating the occurrence of nitrogen-fixation. To count nodules we separated the roots from the plant and scored for the numbers of nodules produced using the following categories: <5, 5-10, 10-25, 25-50, 50-100. For analysis the numbers of nodules produced for each seedling were assigned the mid-point in each category (e.g. 2.5, 7.5, etc).

4.3.5 Statistical analysis

We analysed our data using mixed models fitted within a Bayesian framework. This allowed us to include ‘site’ as a random effect, which we did as a final precaution to control for any differences that arose due to site-specific variation in soil type rather than the presence and identity of the host species, and to estimate mean nodulation and growth rate taking into account this nestedness in our design. Site means were modelled as drawn from normal distributions with mean zero and variances estimated from the data. We assigned the overall intercept and regression coefficients normal prior distributions with mean 0 and variance 1000, and for the ‘site’ term we specified a non-informative uniform prior (0-100) on the standard deviations following Gelman (2006).

We analysed our data in two stages. First, we included either growth or nodulation as the response variable and had four categorical treatment variables: host presence / absence (Host+/-), *Acacia* seedling species, *Acacia* host species (origin) and country. This allowed us to examine how patterns of both growth and nodulation varied between each of the treatment combinations. Each variable was modelled as a two or three level factor variable, including all interactions. Each treatment variable was included in the model by coding them as dummy variables and choosing one of the classes as a reference class with coefficient set to zero. We then calculated the mean growth rate and mean nodulation for each treatment combination, having accounted for site effects. We did not include the sterile controls in the analysis as our central questions relate specifically to relative differences between the Host+ and Host- soils in each country and between species, rather than absolute growth and nodulation values. However, for visual comparison of performance in the experimental soils relative to performance in sterile soils we present the overall mean values for growth and nodulation of each species in the results section.

Second, in order to directly examine the influence of nodulation on plant growth in each range we again set growth as the response variable but this time included nodulation as a continuous variable in a model that included all other treatment variables and their interactions.

Models were fitted using Markov chain Monte Carlo methods as implemented in OpenBugs (Thomas *et al.* 2006) called from the BRugs library in R v. 2.13.1 (R Development Core Team 2011). We ran three chains each with a burn in of 10000 iterations, which were checked for convergence. The posterior distributions were then sampled from a further 10000 iterations of each chain.

For each analysis, we tested the overall effect of each treatment on growth or nodulation by calculating the difference between the two classes in each treatment having the most extreme coefficient values. We calculated this difference for each of the 30000 iterations (10000 from each chain) and then calculated the median and 95% credible intervals of these differences. We considered that imposition of a treatment had a significant effect on growth or nodulation if the 95% credible intervals of the differences did not overlap zero, implying a significant difference in growth or nodulation between at least two classes in that treatment.

To visualise the relationship between growth and nodulation between all of the treatments we calculated the slope of the line that described the influence of nodulation on growth i.e. the incremental increase in growth that resulted from the addition of one nodule under each of the treatment combinations.

4.4 Results

4.4.1 Growth

Seedling growth rate was consistently higher in experimental soils than in the sterile controls in both Australia and New Zealand (Figure 4.1). There was a significant interaction between the presence or absence of an *Acacia* host and country (Figure 4.3a), with seedlings growing more than twice as fast in Host+ soils than Host- soils in New Zealand (0.013 g day^{-1} compared to 0.005 g day^{-1}, respectively), while in Australia growth rate was not strongly influenced by the presence of an *Acacia* host. There were no other significant interactions, indicating that species did not vary in their performance in the Host+ soils of conspecifics or congeners within each country, nor did they vary in the relative difference in growth rate between the Host+ and Host- soils.

4.4.2 Nodulation

Seedlings formed effective nodules in all experimental soils (Figure 4.2), with the exception of the Host- soils from two *A. dealbata* sites in New Zealand, where some seedlings failed to form nodules or formed only non-effective nodules. Around 40% of sterile controls formed nodules, usually forming only one or two with the exception of one *A. dealbata* and one *A. pravissima* seedling in Australia that each formed 10-25 functional nodules, highlighted by the slightly higher mean values for the sterile controls of these species in Australia (Figure 4.2).

As with plant growth rate, there was a significant influence of the presence or absence of an *Acacia* plant host and this influence varied between Australia and New Zealand. In New Zealand, seedlings grown in soils taken from underneath an *Acacia* plant host formed over three times as many nodules as those grown in soils taken in the absence of any *Acacia* (on average 29 compared to 9, respectively). In Australia there was a tendency to lower nodulation in the Host- soils than in the Host+, but this effect was not significant (an average of 18 nodules were formed in the Host+ soils compared to 11 in the Host-).

In contrast to plant growth response, the influence of host presence or absence on seedling nodulation also varied depending on the seedling species and the host species (origin), i.e. there was a significant host × species × origin interaction (Figure 4.3b). This was due to *A. dealbata* seedlings varying in their response to host presence, depending on the host.

Seedlings showed the greatest increase in the number of nodules produced in the Host+ soils of *A. pravissima* when compared to the Host- soils from the same sites (on average, 65 nodules compared to 25, respectively) and the least increase in the Host+ soils of conspecifics when compared to the Host- soils from the same sites (on average, 39 versus 20, respectively). There were no further influences of seedling species, host species or country.

The importance of nodulation for plant growth was confirmed by the significant effect of nodulation on growth rate when it was included as a variable in the model (Figure 4.4). However, this model highlighted that factors other than nodulation also contributed to the variation in growth we observed, because the interaction between Host+/- and country remained significant. In addition, there was some indication that nodule effectiveness for growth varied depending on the seedling species (host × spp × nodulation interaction in Figure 4.5b) and the identity of the host species (host × origin × nodulation interaction). The interaction with seedling species seemed due to *A. baileyana* showing a more marked growth response to increasing nodulation in all soils than the other two species, as well as a more marked increase in the influence of a nodule on growth between the Host+ and Host- soils. Specifically, each nodule formed by *A. baileyana* seedlings in the Host- soils resulted in a 0.0002 g day^{-1} greater increase in growth rate than those formed in the Host+ soils, compared to an increase of 0.0001 g day^{-1} shown by *A. dealbata* and 0.00008 g day^{-1} shown by *A. pravissima*. The interaction with soil origin seemed due to all seedlings showing a limited growth response to increasing nodulation in the soils taken from *A. pravissima* populations. There were no further interactions between nodulation and the other variables examined.

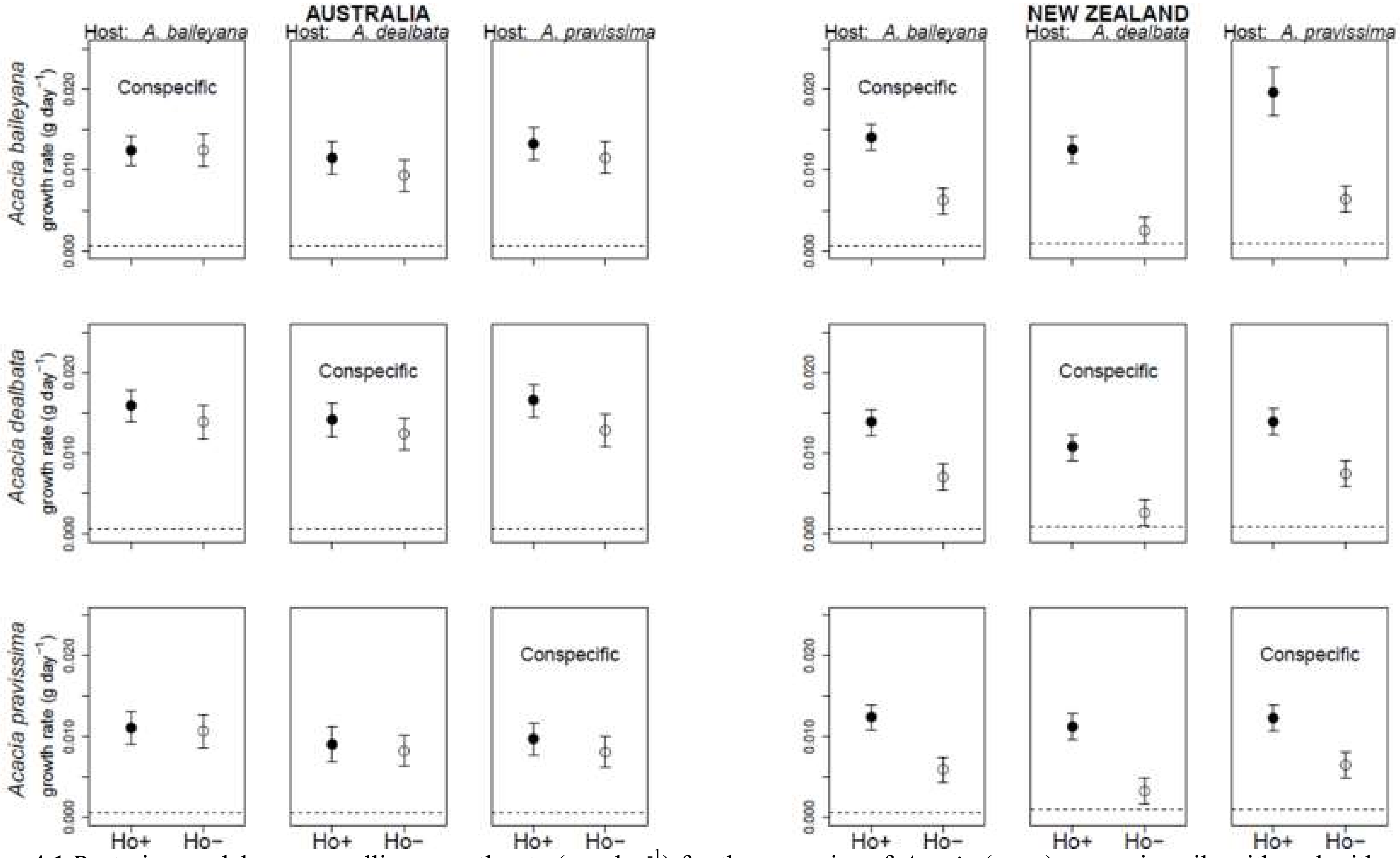

Figure 4.1 Posterior model mean seedling growth rate (mg day^{-1}) for three species of *Acacia* (rows) grown in soils with and without an *Acacia* host (Ho+/-) from sites in the native range (Australia) compared to the introduced range (New Zealand). Each species was grown in soils taken from under conspecifics and the two congeners (columns). Bars represent 95% credible intervals. The dashed line represents mean growth rate of seedlings of each species grown in the sterile controls, which was used as the reference class.

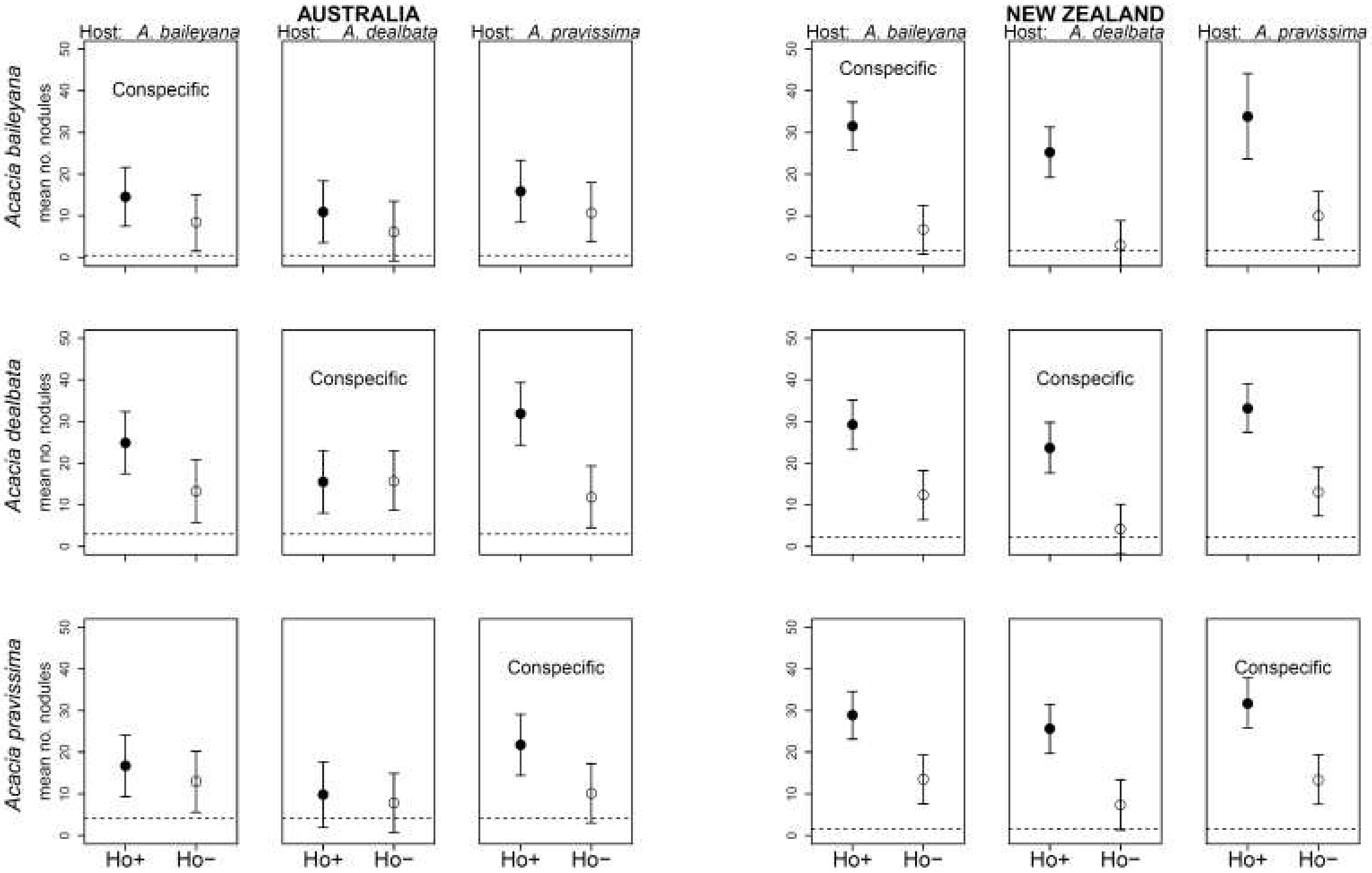

Figure 4.2 Posterior model mean number of nodules produced per seedling for three species of *Acacia* (rows) grown in soils with and without an *Acacia* host (Ho+/-) from sites in the native range (Australia) compared to the introduced range (New Zealand). Each species was grown in soils taken from under conspecifics and the two congeners (columns). Bars represent 95% credible intervals. The dashed line indicates the mean number of nodules formed by each species in the sterile controls, which was used as the reference class.

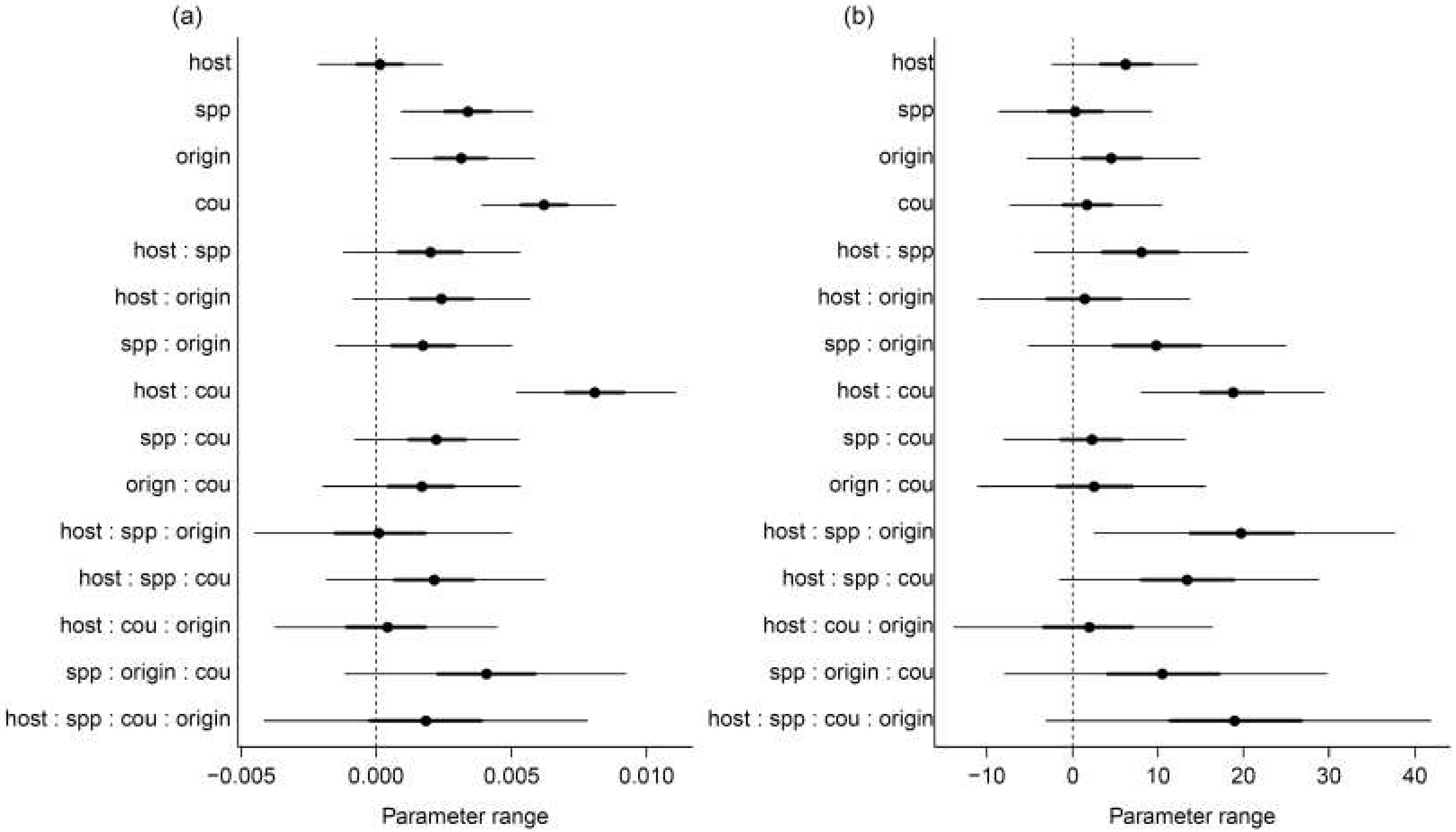

Figure 4.3 The mean and 95% credible intervals for the parameter estimates describing the effect of explanatory variables on growth (a) and nodulation (b). Host: Host+/-; spp: seedling species; cou: country; origin: host species.

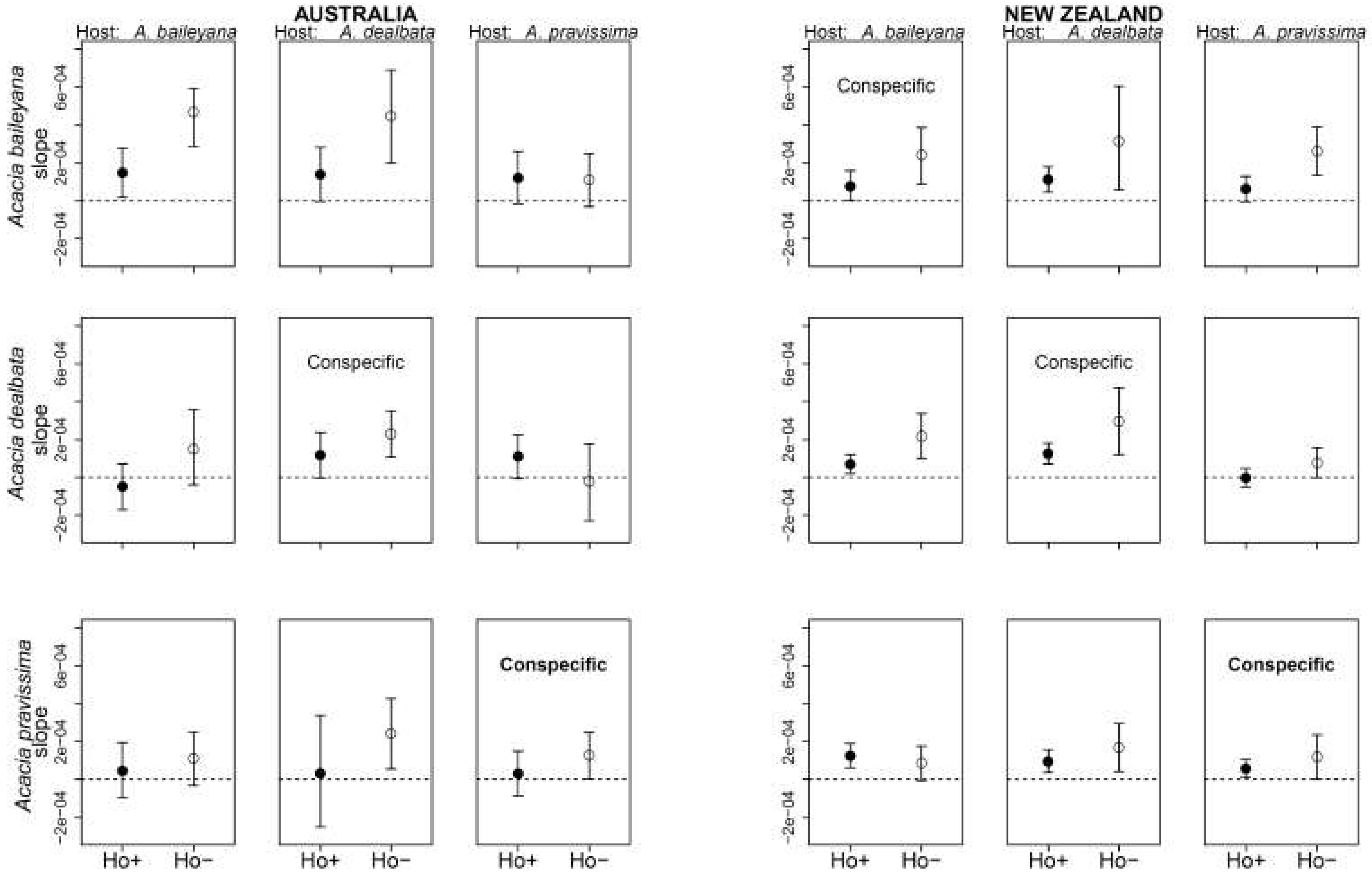

Figure 4.4 The relationship between growth and nodulation for seedlings of three species of *Acacia* (rows) grown in soils with and without an *Acacia* host (Ho+/-) from sites in the native range (Australia) compared to the introduced range (New Zealand). Each species was grown in soils taken from under conspecifics and the two congeners. Bars represent 95% credible intervals. If credible intervals do not overlap zero then higher numbers of nodules can be considered to have a significant positive effect on seedling growth.

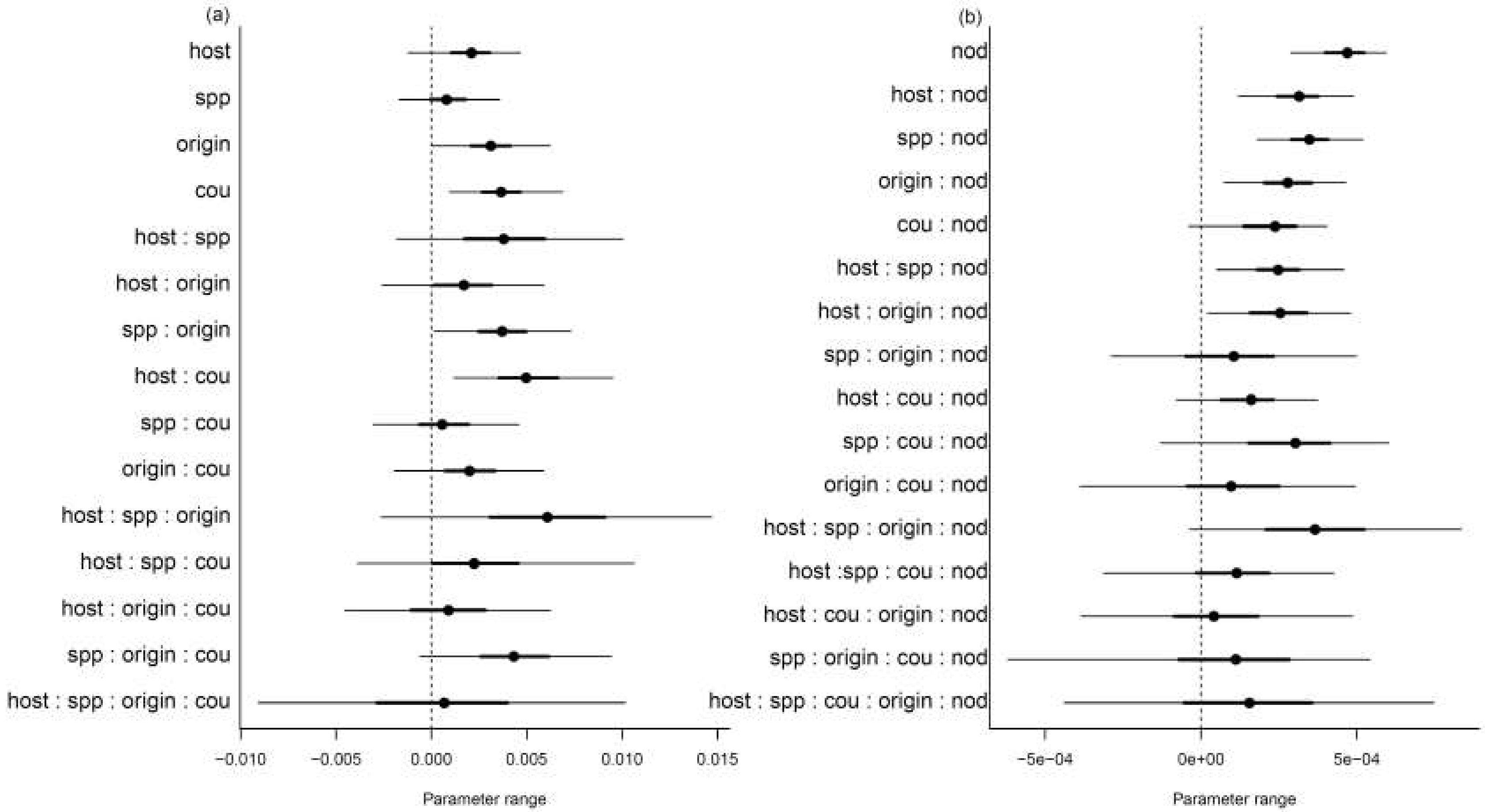

Figure 4.5 The mean and 95% credible intervals for the parameter estimates describing the effect of explanatory variables on growth when nodulation is included as a continuous explanatory variable in the model. Host: host presence/absence; spp: seedling species; cou: country; origin: host species; nod: number of nodules.

4.5 Discussion

We aimed to determine the extent to which positive interactions with soil biota, and more specifically rhizobia, could influence species spread in new locations. Although an increasing number of studies examine the role of soil biota in plant invasion, this is the first to do so in the native and introduced range and across species that vary in invasion success. We found support for our first hypothesis: seedlings showed a greater reduction in performance away from established conspecifics in their introduced New Zealand relative to their native Australian range. However, we did not find support for our second hypothesis: species did not vary in the extent to which they were limited by rhizobial availability away from established conspecifics. We found support for our final hypothesis, with all species performing equally well in soils from beneath congeners.

4.5.1 Rhizobial availability limits plant performance in the introduced relative to the native range

Our findings in Australia suggest that population spread would not be constrained by a lack of rhizobia at sites away from established individuals and that this is partly due to the widespread availability of rhizobia in the region (Barnet & Catt 1991). Although there was some indication that rhizobial availability was reduced away from conspecifics, which we might expect as populations can decline in the absence of a plant host (Parker 2001; Thrall *et al.* 2005), this was not significant and did not appear to incur a significant reduction in plant performance. Other studies that have examined the influence of soil biota on species performance have found that interactions with natural enemies in the soil are generally more pronounced in species' native ranges (e.g. Klironomos 2002; Reinhart *et al.* 2003; Reinhart & Callaway 2006), particularly in association with conspecifics (MacKay & Kotanen 2008). While we were unable to separate the positive and negative interactions acting on seedlings, due to using whole soils that contain the suite of organisms present at any location, plant performance in the experimental soils compared to the sterile soils demonstrated that positive interactions were the dominant influence on plant performance and there was no suggestion in our data that seedling performance was reduced in association with conspecifics. It also seems unlikely that that the actions of soil pathogens confounded our results, since growth responses largely followed nodulation and the influence of nodulation on growth was similar in each range.

In contrast to Australia, our results suggest that population spread in New Zealand could be constrained by low availability of rhizobia and indicate individuals dispersing away from established populations would experience a significant reduction in performance. Although seedlings showed some degree of nodulation in most soils in New Zealand, plant performance and nodulation was reduced away from *Acacia* plant hosts, indicating that compatible rhizobia were present at much lower densities. While consistent with studies that have found *Acacia* nodulating throughout their introduced range, this finding demonstrates that rhizobia are a limiting resource for *Acacia* introduced to new locations. Although this supports work that has demonstrated limitation in introduced *Cytisus scoparius* (L.) Link and *Chamaecrista fasciculate* (L.) Moench in the United States (Parker *et al.* 2006; Stanton-Geddes & Anderson 2011), it is in contrast to research that showed *Robinia pseudoacacia* L. performed equally well in its invaded European as its native American range (Callaway *et al.* 2011). The conflicting findings could be due to differences in symbiotic promiscuity between the taxa used in each study, or to their ability to share the same rhizobia as native or other introduced legumes (Parker *et al.* 2006). In New Zealand, alien and native plant species have been found to nodulate with phylogenetically distinct rhizobia (Weir *et al.* 2004; Weir 2006), but where alien species share rhizobia with native plants, limitation may be less likely and patterns more similar to those found in Australia might occur. In addition, since this is the first examination of plant performance in natural soils from both established and uncolonised sites in both the native and introduced ranges, it is the first direct test of limitation for seedlings arriving in new locations.

4.5.2 The role of rhizobia in plant invasion

Our finding that there was no species-specific variation in plant performance in either the native or introduced range suggests that symbiotic associations in the native range may be a good predictor of symbiotic success in the introduced range, but are not a good indicator of invasion success as has been suggested (Rodríguez-Echeverría *et al.* 2011). In addition, although symbiotic promiscuity is expected to confer invasion success (Richardson *et al.* 2000a), we found no evidence that a higher symbiotic promiscuity might explain the invasive success of *A. dealbata* relative to the other species we examined, since all species showed equal limitation away from established conspecifics. Although there was some variation in the influence of nodulation on growth, this was limited and could not explain the variable invasion success of the three species. Thus, interactions with rhizobia and soil biota in general are not driving the variable invasion success shown by *Acacia* in New Zealand.

Although interactions with rhizobia do not seem to drive invasion success, the increase in performance associated with an increase in nodulation highlights their importance for initial establishment, which could be facilitated by association with a congener. The presence of native or naturalised congeners is expected to facilitate the establishment and invasion of other species (Richardson *et al.* 2000a; Dickie *et al.* 2002; Hill & Kotanen 2012) and our data indicate that species introduced to locations where *Acacia* are already present, irrespective of the species, may have significantly greater chances of establishment than those introduced to areas with no *Acacia*. In addition, it suggests that this influence is likely to occur over relatively short timescales, as seedling performance for all three species was the same in the Host+ soils of garden plantings of the more recently introduced *A. pravissima* as in the Host+ soils of large, established populations of *A. dealbata*.

However, the absence of any influence of host species also provides further evidence that interactions with rhizobia and soil biota in general are unlikely to be important for *Acacia* invasion. It has been suggested that interactions between rhizobia and alien *Acacia* might mirror that shown between pines (*Pinus* spp.) and their fungal symbionts in the southern hemisphere (Parker 2001; Parker *et al.* 2006; Rodríguez-Echeverría *et al.* 2011), with species showing initial limitation that is overcome after a lag phase as populations of the necessary symbionts build up over time (Richardson *et al.* 2000a; Parker 2001; Parker *et al.* 2006; Pringle *et al.* 2009). That our study species showed similar growth limitation in the Host- compared to the Host+ soils at sites with large, well-established populations of *A. dealbata* as they did at those with just a few ornamental *A. pravissima* trees suggests that if such a lag phase exists, it is unlikely to influence species spread. In addition, it highlights that any facilitative effect of association with native or naturalised congeners would likely be highly localised.

4.5.3 Additional influences

There was a considerable amount of unexplained variation in growth rate present in our data even when nodulation was included in the analysis. This is likely partly due to natural variation in seedling fitness and in the nutrient content of soils underneath focal trees, but also the potential contribution of other soil biota to overall plant performance (Klironomos 2002; Rodríguez-Echeverría 2010). Additional interactions that might influence plant performance include those with soil pathogens and mychorrizal fungi. Again, however, that plant growth response was consistent across all three species indicates that regardless of the mechanism underlying the patterns we found, interactions with soil biota do not appear to underpin the variable invasion success of the three species we examined.

4.5.4 Future directions

In order to measure rhizobia-mediated limitation to alien plant spread we focused on three species that have all naturalised, expecting that in order to establish species would have encountered compatible rhizobia but that their spread may be limited by their ability to associate with them in locations away from established trees. However, the species we examined all showed similar abilities to associate with the rhizobia present when colonising new sites. Future work that examines species that are either confirmed to show low promiscuity or have completely failed to establish in new locations may reveal a more important role of soil biota in determining patterns of naturalization. Furthermore, in New Zealand we conducted our study in highly modified environments, because we were examining the potential for interactions with rhizobia to constrain initial spread away from introduction sites. Since this may have increased the likelihood *Acacia* encountered compatible rhizobia associated with other alien plant species, conducting future studies across a greater variety of habitats, including more intact ecosystems as well as those that are highly modified, might provide further insights into the mechanisms underpinning plant invasion.

By comparing species' abilities to form associations when first introduced to an area in both their native and introduced range we have shown that the availability of rhizobia has the potential to limit the spread of introduced *Acacia*. Although the presence of established congeners may facilitate establishment, this influence was also highly localised. In addition, by examining species that have failed to successfully spread as well as those that have become invasive we have demonstrated that interactions with rhizobia and soil biota in general did not explain the differential success these species have shown since introduction to New Zealand. This suggests that the role of soil biota in alien species' spread may not be as important as previously thought.

Chapter 5 - Discussion

In this thesis I aimed to determine whether variation in species' abilities to form mutualistic interactions can help explain invasion outcomes. Specifically, I examined whether interactions with pollinators, seed dispersers and belowground symbionts can explain the variable invasion success of *Acacia* introduced to New Zealand. I focused on three species that differ in the extent to which they have established and spread since introduction: *A. dealbata*, *A. baileyana* and *A. pravissima*. I examined whether the strength of mutualistic interactions and the potentially mediating role of antagonistic interactions varied for species in their introduced New Zealand range relative to their native Australia range (country effect), and whether the extent of this differed between species (country × species effect). This is the first study to have directly measured mutualistic interactions in both the native and introduced range and across species that have shown varying invasion outcomes. I found support for the widespread assumption that mutualistic interactions are generalised and demonstrated that species introduced to new locations are able to establish mutualistic interactions with pollinators and dispersers to the same degree as in their native range. In contrast, I found that plant performance is limited by rhizobial availability for species colonising new sites in New Zealand but not in Australia. Overall, I found no evidence that mutualistic associations could explain the variable invasion outcomes of each species.

5.1 Main Results

5.1.1 Are mutualistic interactions with pollinators an important determinant of plant success?

In Chapter 2 I quantified the reproductive success and seed output of each species in New Zealand relative to their native range in Australia. Factors that influence seed output, such as pollination and predispersal seed predation, are considered particularly important for plant invasion (Pyšek & Richardson 2007) because they influence local establishment. They can also indirectly influence spread into new locations by controlling the number and quality of propagules for dispersal. I found a significant species × country interaction for three variables: predispersal seed predation, percentage viable seeds per pod and overall seed rain. This was due to *A. pravissima*, currently only considered as casual in New Zealand, demonstrating lower rates of seed predation and higher seed output relative to its native range in Australia, and relative to the other two species. There was no significant variation in the success of pollination either between the two ranges or among species. Therefore, my findings support the prediction that species with a generalised pollination syndrome are unlikely to be pollen limited in new locations (Richardson *et al.* 2000a; Stout *et al.* 2006; Gibson *et al.* 2011), and suggest that this does not explain the variable invasion success shown by *Acacia* in New Zealand.

My approach in this chapter was to focus on the broad patterns of reproductive success, since overall seed output is ultimately most important for plant invasions. However, many other studies that examine the importance of pollination for plant invasion focus on more specific details, such as identifying morphological traits that might enhance pollination success in new locations (Bjerknes *et al.* 2007; Küster *et al.* 2008; Vanparys *et al.* 2008), or examining the influence of breeding syndromes on plant invasion (van Kleunen & Johnson 2007; Burns *et al.* 2011; Petanidou *et al.* 2012). Although the flowers of the three species I examined are morphologically very similar, making floral trait variation unlikely to be important between species, factors that could potentially lead to variation in reproductive success between *Acacia* species include levels of self-compatibility and the timing of flowering.

Invasive plant species are expected to be more likely to show self-compatibility in their introduced than in their native range, and relative to non-invasive plant species (van Kleunen & Johnson 2007; Petanidou *et al.* 2012). There is some indication that *A. dealbata* is self-compatible in its introduced South African range but not in Australia (Broadhurst *et al.* 2008; Rodger 2011). The comparable pollination success I recorded between New Zealand and Australia could therefore be due to either similarity in the effectiveness of pollination between the two countries, for example due to the widespread introduction of generalised pollinators, or to an increase in self-compatibility in the introduced range buffering species against a reduction in the effectiveness of pollination. For this reason, examining levels of self-compatibility for *Acacia* in each country may have provided more information as to the mechanisms underpinning the broad patterns I recorded. However, as neither pollen limitation nor overall reproductive output determine the variable success of the three species I examined, it is unlikely that research into the breeding systems of *Acacia* in their introduced and native ranges would further our understanding of why some *Acacia* are so invasive where others are not.

Similarly, flowering phenology and the duration of flowering are expected to influence the effectiveness of pollination in new locations (Rejmánek 1999; Lake & Leishman 2004) and may contribute to the success of introduced *Acacia* (Gibson *et al.* 2011). For example, the three species all flower in winter when resources for pollinators are more scarce, and *Acacia* species in general have long-lived flowers. More detailed observations of floral visitors could therefore reveal whether the pollination success of *Acacia* in New Zealand is due to their ability to attract the available pollinators to a greater degree than neighbouring plants. However, again the absence of species-specific variation in pollination success and seed output indicate that such research would be unlikely to highlight an important role of flowering phenology in the variable invasive success shown by different *Acacia* species.

5.1.2 Are mutualistic interactions with seed dispersers an important determinant of plant success?

In Chapter 3 I quantified the probability of seed removal for each species in New Zealand compared to their native ranges in Australia, examining both invertebrate and vertebrate removal. Seed dispersal may be an important determinant of invasion success because it facilitates the colonisation of new sites away from the parent tree, influences the habitat seeds arrive into and can protect seeds from further predation (Wenny 2001; Giladi 2006). In myrmecochorous *Acacia* it is also likely important in building up seedbanks that may contribute to their success (Richardson & Kluge 2008). I found a significant difference in rates of seed removal by invertebrates between countries with overall seed removal lower in New Zealand than in Australia. The pattern of removal indicated that this was due to lower levels of seed predation in New Zealand and that seed removal associated with dispersal was similar between the two countries. I did not find a significant species × country interaction. Therefore, although my findings indicate that myrmecochory is diffuse such that species introduced into regions with a depauperate ant fauna can establish dispersal mutualisms, neither interactions with seed dispersers nor the influence of seed predators could explain the relative success of the three species.

As with Chapter 2 my approach was to identify broad patterns that might explain the differential success shown by species in New Zealand. The scale of doing so meant that it was not possible to conduct more targeted research to tease apart the mechanisms underlying the removal patterns I recorded. For example, it is possible that the effectiveness of associations differ between the two ranges due to the smaller size of ants likely to be present in the survey area in New Zealand (Table 3.2), or because dispersal by seed specialists is more effective than dispersal by generalist foragers. Approaches such as following the fate of seeds removed and the habitat they were deposited into, or identifying the ant species involved in removal (e.g. Auld 1986a; Hughes & Westoby 1992b; Ireland & Andrew 1995), could therefore have provided more information on the effectiveness of dispersal. However, because the ant fauna of New Zealand is relatively depauperate only a few ant species are likely to have been involved in seed removal, meaning that the seeds of each *Acacia* species were probably removed by the same ant species, leading to similar fates. For this reason, even if seed dispersal by ants is more effective in species' native ranges in Australia, a reduction in effectiveness is unlikely to underpin the variable success of species in New Zealand.

My findings within this chapter highlight the importance of considering the potentially mediating role of natural enemies when examining mutualistic interactions. Since ants only move seeds short distances it may be unlikely that myrmecochory is strongly associated with population spread in *Acacia* and the advantages may be more linked to protection from predation, directed dispersal and vertical movement into the seedbank. My finding that predation by both vertebrate and invertebrate seed predators is low in New Zealand indicates that even without effective dispersal, a large proportion of seeds could be incorporated into the seedbank over time and small-scale wind dispersal combined with low predation rates may compensate for any reduction in the quality of dispersal that was not identified within my study.

5.1.3 Are mutualistic interactions with rhizobia an important determinant of plant success?

In Chapter 4 I examined the extent to which rhizobia could limit plant performance away from established individuals in each country, and whether variation in species' abilities to overcome any limitation might underpin their variable invasion success. I also extended this to determine the potential for established congeners to facilitate species' colonisation in new locations. The potential importance of rhizobia for plant invasion is less well understood than pollination and dispersal. Although alien legumes have been recorded to nodulate with the available rhizobia (Rodríguez-Echeverría 2010; Callaway *et al.* 2011; Birnbaum *et al.* 2012), there is some evidence that rhizobia are limiting at early stages of colonisation (Parker *et al.* 2006). The availability of rhizobia in new locations may therefore be an important barrier to species' spread outside of cultivation. Here, I found a significant country effect and species experienced rhizobial limitation of plant performance away from established *Acacia* in New Zealand, but in Australia rhizobia appeared to be more widespread and plant performance was consistent across all soil treatments. I did not find a significant species × country interaction. Interactions with rhizobia were therefore important for species establishment but could not explain the relative success of the three species in New Zealand.

This chapter again highlights both the advantages and disadvantages of undertaking such broad comparative studies. Although other studies that have examined the importance of rhizobia for plant invasion have found that *Acacia* readily nodulate where introduced, they usually examine plant performance in soils that were experimentally inoculated with rhizobia or were taken from underneath established conspecifics (Rodríguez-Echeverría 2010; Birnbaum *et al.* 2012; Boukhatem *et al.* 2012), with only rare examples of species planted into soils that did not previously contain *Acacia* (Weir 2006). Similarly, studies that have examined interactions between rhizobia and species in other genera have also focused on nodulation in soils taken from underneath a conspecific host or those experimentally inoculated with rhizobia (Parker *et al.* 2006), or in soils taken away from any conspecifics (Callaway *et al.* 2011). Only two studies have examined species nodulation in both their native and introduced range (Birnbaum *et al.* 2012; Ndlovu *et al.* 2013), and no studies have examined species that vary in invasiveness. Therefore, my data represent the most complete overview of alien plant performance and nodulation with rhizobia.

By taking this overview I have been able to demonstrate that rhizobia can be limiting for alien plant species and could constrain their ability to spread away from introduction sites, as well as that interactions with rhizobia, and soil biota in general, cannot explain invasion outcomes. However, a disadvantage of this broad approach is that it is again difficult to identify the mechanisms underlying the patterns I recorded. For example, it has been suggested that the ability of *Acacia* to nodulate with rhizobia in New Zealand is due either to the background presence of cosmopolitan rhizobia in New Zealand, or because rhizobia are introduced along with the plant (Weir 2006), which has been suggested in other systems (Rodríguez-Echeverría 2010; Birnbaum *et al.* 2012). The only way to elucidate this would be to adopt the approach taken by Birnbaum *et al*. (2012) and characterise the rhizobia associated with plant species in both the native and introduced range. Similarly, quantifying the density of rhizobia available would be useful as it would enable more accurate conclusions to be drawn on the contribution of rhizobia to plant performance. Although, as with previous chapters, my findings indicate that such research would be unlikely to identify a role for rhizobia in influencing invasion outcomes for *Acacia* in New Zealand, our current understanding of the processes that could facilitate alien species forming mutualistic associations with rhizobia in new locations is more limited than for pollination (generalised pollination syndrome, floral similarity to native species) and dispersal (the diffuse nature of dispersal mutualisms).

5.2 Additional influences and the role of mutualisms

Plant species are subject to a variety of biotic and abiotic influences, which may all influence their invasive ability. In addition, human factors are frequently identified as important contributors to invasion success (Křivánek *et al.* 2006; Pyšek *et al.* 2009b; Wilson *et al.* 2009; Castro-Díez *et al.* 2011; McGregor *et al.* 2012). Influences other than those I have examined in this thesis could be more important for the invasive success of *Acacia* species, could confound attempts to identify a role of mutualistic interactions, or could act in synergy with mutualistic interactions to determine invasion outcomes.

5.2.1 The role of other biotic interactions

By including data on the influence of natural enemies where possible, my findings indicate that interactions with natural enemies at each of the stages I examined are also unlikely to determine the variable invasion success shown by the three species. However, additional interactions with the biota present in new locations may influence invasion incomes.

A potentially important influence on alien plant spread is that of seedling herbivores. Seedling herbivory was demonstrated to limit the invasion potential of *Cirsium vulgare* (Savi) Ten. in the USA (Eckberg *et al.* 2012) and of *Pinus radiata* D. Don in Chile (Becerra & Bustamante 2008), while invasive alien species were found to experience higher leaf damage than non-invasive plant species in the USA (Cappuccino & Carpenter 2005). Herbivore mediated biotic resistance to plant invasion is commonly used to explain the failure of some plant species to invade (Maron & Vilà 2001). Biotic resistance resulting from competitive interactions with local flora is also predicted to influence invasion outcomes, potentially to a greater degree than herbivory (Levine *et al.* 2004).

It is possible that variation in seedling herbivory is more important for *Acacia* spread away from introduction sites. If so, then this could interact with the three mutualisms I examined to determine invasion success. Although neither pollination nor seed dispersal varied between the three *Acacia* species (Chapters 2 and 3, respectively), high propagule pressure resulting from successful pollination and dispersal could influence the strength of seedling herbivory and competitive interactions, while variation in species' susceptibility to herbivory and their ability to compete with native flora may influence invasion outcomes (D'Antonio *et al.* 2001). Similarly, the reduced performance resulting from the rhizobial limitation away from conspecifics I identified in Chapter 4 could have a strong influence on species' competitive ability or the extent to which they can tolerate seedling herbivory, and the importance of this may vary between the three species.

5.2.2 Additional differences between the species

Congeneric comparisons are often considered useful because they allow for some degree of control over differences in phylogeny and life-histories (Agrawal & Kotanen 2003; Burns 2006; Muth & Pigliucci 2006; Powell *et al.* 2011). In addition, both floral and seed morphology were similar between the species I examined allowing me to ask whether species varied in the extent to which they were able to establish mutualistic interactions, rather than whether floral morphology or dispersal syndrome influence invasion success. However, there were inevitably differences between species that could not be controlled for and may influence invasion success. For example, the three species differ in their maximum height, phylogenetic relatedness and leaf morphology.

Plant height was found to be a good predictor of *Acacia* invasive success in South Africa (Castro-Díez *et al.* 2011) and globally (Gallagher *et al.* 2011). One reason is that tall trees are more likely to be used for forestry and thus more widely planted, leading to an increase in propagule pressure. Alternatively, it could be due to dispersal benefits because plant height is positively correlated with dispersal distance (Thomson *et al.* 2011), which is important in population spread. Since my findings within Chapter 3 indicate that all three species establish dispersal mutualisms and escape seed predation, both underneath conspecifics and away from them, the greater height of *A. dealbata* combined with some dispersal and little predation could contribute to its invasive success, allowing it to both spread its seeds and build up seedbanks over a wider area than the other two species.

In terms of the potential role of phylogeny, *A. dealbata* and *A. baileyana*, both more successful than *A. pravissima* in New Zealand and other parts of the world, are more closely related to each other than to *A. pravissima* (Miller *et al.* 2011). These two species are also morphologically more similar to each other than to *A. pravissima*, being of the bipinnate leaf form rather than phyllodinous. Other variables related to phylogenetic or morphological similarity may therefore be responsible for variation in invasive success and could suggest I should have chosen species that were more similar. For example, there is some evidence that species that are phylogenetically more similar are more likely to share specialist natural enemies (Strauss *et al.* 2006). However, my findings indicate that *A. pravissima* is the least likely to encounter natural enemies in New Zealand, and records of natural enemies are lower for this species in general (Chapter 2). In addition, in *Acacia* there is no evidence that phylogenetic relatedness or other functional traits related to morphological differences between the species, such as specific leaf area, is correlated with invasiveness (Gallagher *et al.* 2011; Gibson *et al.* 2011; Miller *et al.* 2011) despite evidence that they may be important in other systems (Lake & Leishman 2004).

5.2.3 The role of native range size and varying habitat requirements

Native range size often correlates with plant invasiveness (Agrawal *et al.* 2005; Pyšek *et al.* 2009a; Castro-Díez *et al.* 2011). Species with a large native range size are expected to show greater invasive success either because they can tolerate a wider range of abiotic and biotic variation, or because they have an increased likelihood of human encounter and selection (Agrawal *et al.* 2005; Pyšek *et al.* 2009a). Large native range size could therefore indicate a broader tolerance of environmental variation, which may be more important than mutualistic interactions, or imply that species are more likely to establish mutualistic interactions in new locations.

The native range size of the three species I used in this study correlates with their invasive success both in New Zealand and globally (Castro-Díez *et al.* 2011; Richardson & Rejmánek 2011), meaning differences in habitat requirements or factors associated with selection for planting could be more important determinants of their invasive success. However, native range size in *Acacia* may be more important in terms of introduction and naturalisation than invasion (Hui *et al.* 2011). In addition, my data indicate that a broader environmental tolerance does not underlie the variable success shown by the three species in New Zealand. *Acacia pravissima*, the least widespread species, exhibited the greatest release in New Zealand where natural enemy attack was lower, seed production was higher and trees were larger than in its native range in Australia. There was also no evidence that soil conditions might disadvantage this species and it showed comparable performance in all soil types as the more widespread *A. dealbata* and *A. baileyana* (Chapter 4).

5.2.4 The role of propagule pressure and planting time

A potential problem with field studies of plant invasion is the disproportionate influence of human-mediated factors in determining species' invasion success, which could confound any attempts to elucidate biotic drivers of invasion (Pyšek & Richardson 2007). In particular, propagule pressure (Richardson *et al.* 1994; Křivánek *et al.* 2006; Dawson *et al.* 2011; McGregor *et al.* 2012) and time since introduction (Křivánek *et al.* 2006; Pyšek *et al.* 2009b) are frequently identified as important determinants of invasion success and human influences were found to be the best predictors of the range and abundance of alien *Acacia* in South Africa (Castro-Díez *et al.* 2011).

The three species vary in terms of residence time, which correlates with their invasion success in New Zealand, although there is no accurate introduction date for *A. pravissima* (Table 1.1). In addition, *A. dealbata*, a highly invasive species in New Zealand, has also been more intensively planted for use in agriculture and forestry. This could indicate that introduction date and planting effort are indeed more important determinants of *Acacia* success in New Zealand. For these reasons, choosing species that have comparable planting dates yet have shown differential invasive success could have been a stronger test of the importance of biotic interactions. However, all of the species that were introduced in the 1800s were also widely and intensively planted for forestry, are invasive in New Zealand and considered environmental weeds (Table 1.1) and, of the other species, no suitable populations were located within the study area.

Nevertheless, mutualistic interactions may interact with human factors to determine invasion outcomes. One way in which this might happen is if plant species acquire mutualists over time (Pyšek *et al.* 2011), which has also been demonstrated between plant species and their natural enemies (Diez *et al.* 2010; Wingfield *et al.* 2011) and may contribute to the lag phase frequently seen in plant invasions (Sakai *et al.* 2001; Aikio *et al.* 2010). However, it seems unlikely in the case of *Acacia* in New Zealand as all three species appear to have equally established mutualistic interactions. Alternatively, long-distance dispersal events may be a key constraint for *Acacia* invasion in New Zealand. Human-mediated dispersal not only facilitates the colonisation of areas outside species' native ranges, but often results in repeated introductions to multiple sites in those new ranges (Wilson *et al.* 2009). Repeated human-mediated long-distance dispersal events combined with local reproduction and spread could therefore be important for *Acacia* invasion. Chapters 2 and 3 both highlight that neither reproduction nor dispersal and predation limit species at local scales, and high propagule pressure could compensate for reduced plant performance related to rhizobial availability (Chapter 4).

5.2.5 Congeneric facilitation

Darwin's naturalization hypothesis posits that introduced species are less likely to establish into regions with native congeners as they are more likely to come under attack from native antagonists, such as herbivores and pathogens (Darwin 1859). However, by the same premise, alien species with native congeners may be more likely to establish due to similarity in habitat requirements, or the presence of an existing suite of organisms with which to develop mutualistic relationships. It has been demonstrated that species with native congeners are more likely to establish in New Zealand (Duncan & Williams 2002), though the strength of facilitation likely varies according to the stage of invasion and aspects of the invaded community (Diez *et al.* 2008). The presence of naturalised congeners may also influence the likelihood alien plants naturalise when introduced to new regions, particularly if they have a long association with an area.

My findings within Chapter 4 indicate that the presence of established congeners would facilitate establishment at local scales in terms of the availability of rhizobia. It is also possible that the similar floral and seed morphology between *Acacia* means that more recent introductions are able to more readily form mutualistic associations with pollinators and seed dispersers, taking advantage of the mutualisms *A. dealbata* may potentially have built up over time (Pyšek *et al.* 2011). Therefore, congeneric facilitation could explain why there were no differences between the three species I examined in terms of pollination and dispersal, although this could not explain why there were no differences between the species in the extent of rhizobial limitation.

5.2.6 Summary

The variety of research approaches to understanding biological invasions highlight the complexity of studying natural systems. No single factor is likely to determine a species invasion success, nor readily extend to other species and systems. Furthermore, the importance of various influences likely changes as species progress along the continuum from introduced to invasive. Even where factors are identified as potentially contributing to invasive success, direct impacts on population growth must be demonstrated before they can be assumed to influence invasion outcomes.

My findings indicate that neither mutualistic interactions nor the mediating influence of natural enemies determine the variable invasion success of the three species in New Zealand. In addition, they imply that the three species are all sufficiently similar in their mutualistic interactions that these are unlikely to drive invasion outcomes in other regions. However, considering these results in the context of the additional influences plant species are subject to highlights that the patterns of association with mutualists I have identified could interact with those influences to determine invasion outcomes. Furthermore, although influences not controlled for in my study, such as human-mediated propagule pressure, could confound attempts to identify a role of mutualisms in plant invasion, the similarity in mutualistic interactions between the three species I examined makes it unlikely that it could have obscured any important interactions in this study.

5.3 Future research

5.3.1 *Acacia pravissima* as a potential invader

The Australian *Acacia* are an important group of trees worldwide. They are of considerable economic value but the invasive success of many species means they can also incur substantial costs. For this reason, there is a considerable research effort focused on determining what differentiates the invasive species from the non-invasive species. By identifying important stages in their life-cycle further research and management options for their control can be developed. In *Acacia*, the accumulation of large seedbanks is implicated in their success (Richardson & Kluge 2008) and preventing such accumulation is identified as an important focal point for future control because eradication after seedbanks build up is difficult (Gibson *et al.* 2011).

A consistent theme in this thesis is that *A. pravissima*, currently only considered causal in New Zealand, has established effective mutualisms to the same degree as more widespread and invasive species and experiences either greater or equal natural enemy release. In Chapter 2 I also found that this species grows larger and produces more seeds in New Zealand than in Australia, indicating that competition, climate or habitat suitability are unlikely the cause of its limited spread in New Zealand. In addition, since the available pool of potential pollinators and dispersers of this species in New Zealand are relatively limited, and rhizobial availability is low relative to its native range, it is unlikely that the successful establishment and spread of this species in other regions would be limited by mutualistic interactions. Therefore, what is currently preventing its spread in New Zealand, and other parts of its introduced range, is unclear but could include some of the additional influences discussed previously. For example, a longer residence time and an increase in the number of locations this species is planted may increase the likelihood *A. pravissima* encounters suitable habitats for establishment, including sites where compatible rhizobia are abundant. Alternatively, future changes in land use may release seeds currently present in the seedbank, particularly as seedbanks are likely to be extremely large close to source populations (Chapter 2). Land used changes may also increase the availability and spatial distribution of compatible rhizobia in the soil, particularly if species that share rhizobia with *Acacia* are introduced or more widely planted (Chapter 4). Regardless of the potential mechanisms, the findings in this thesis highlight that species of *Acacia* not currently considered as invasive have considerable invasion potential and identifying what factors have so far prevented their establishment and spread on a wider scale should be an important focus of future research.

5.3.2 The unit of comparison: invasive, naturalised or introduced?

Biotic factors are predicted to be more important at later stages of invasion and only indirectly associated with introduction and naturalisation through their influence on selection for planting (Pyšek *et al.* 2009a). However, while my findings indicate that biotic interactions with mutualists and natural enemies do not determine the invasive status of *Acacia* in New Zealand, they could indicate that these interactions are more important in determining which species become naturalised. Species that have successfully naturalised may have more in common with species that are invasive as they have already overcome several important barriers. Although studying species that have failed to naturalise is difficult, since the availability of individuals and populations to study is inherently limited, such research would be a logical next step in terms of understanding the importance of mutualistic interactions in general, and for *Acacia*.

Of the mutualistic associations I examined, interactions with rhizobia may have the greatest influence on naturalisation success. Although my findings in Chapter 4 indicate that interactions with rhizobia do not underpin the variable invasion success of the three species, they do highlight that rhizobia are a limiting resource, and indicate that the three species are similar in terms of their symbiotic promiscuity. Since there is evidence that *Acacia* species vary in their symbiotic promiscuity in their native range (Thrall *et al.* 2000; Thrall *et al.* 2007), species with lower promiscuity than those I focused on may show complete failure to establish into host- soils. For this reason, future work could extend this study to include species that have failed to naturalise and to determine the extent to which variation in species promiscuity might underpin invasion outcomes.

5.3.3 The invasive success of *Acacia* relative to other taxa

Although my findings indicate that more detailed investigation of breeding systems and flowering phenology (Chapter 2), seed fate (Chapter 3) or the identity of rhizobia in the soils (Chapter 4) would be unlikely to determine factors underlying the variable success shown by *Acacia* species in New Zealand, such work may highlight why *Acacia* as a group are so successful. This would not only help with the future management of alien *Acacia*, but could also help identify future invaders in other taxa. If the comparative approach is extended to other taxa, it may also highlight why species for which reproduction is more strongly tied to the three interactions I focused on fail to become invasive. For example, species for which population growth is more seed limited may be more dependent on seed production and dispersal in new locations. In addition, if such species are also leguminous they may also be less likely to overcome rhizobial limitation since local propagule pressure would likely be lower than in *Acacia*. My findings highlight that starting with a broad comparative approach is important to pinpoint potential avenues for further research and should ideally be undertaken before any more detailed research is carried out.

My findings within Chapter 4 not only highlight that rhizobia are limiting for species introduced to new locations, but also suggest a kind of positive feedback over time. Soil feedback studies are increasingly popular (e.g. Klironomos 2002; Agrawal *et al.* 2005; Callaway *et al.* 2011), and usually involve repeated planting of species into field soils under glasshouse conditions to test whether plant performance increases or decreases for later generations when compared to earlier ones. In that way, the Host+ soils of Chapter 4 essentially represent later stage feedback soils under natural conditions. However, while many studies find negative feedback, including one undertaken in New Zealand (Diez *et al.* 2010), my data indicate positive feedback, with performance increasing in soils that have previously supported conspecifics (Host+). The difference in outcomes may be due to the use of leguminous versus non-leguminous species. For this reason, the invasive success of *Acacia* could be due to the generation of a positive feedback loop when colonising new sites, giving them a competitive advantage over other species. Therefore, a potential avenue for future research is to extend the experiment used in Chapter 4 to other taxa, and include both leguminous and non-leguminous species.

5.4 Conclusions

Overall, my findings indicate that mutualistic interactions do not drive the variable invasion success shown by introduced *Acacia* in New Zealand. Since New Zealand represents what should be a limiting environment for *Acacia* in terms of the availability of mutualists, this in turn implies that mutualistic interactions are unlikely to be important for the variable invasion success shown by species introduced to other locations. However, that all three species appear to readily establish pollination and dispersal mutualisms, as well as limited symbioses with rhizobia, indicates that mutualisms could interact with other variables to determine invasion outcomes. Since my findings indicate that species of *Acacia* not currently considered invasive may become so in the future, identifying mechanisms underlying both the variable success of *Acacia* species and the success of *Acacia* in general should be an important avenue for future research.

My findings also highlight the importance of broad geographical and comparative studies when attempting to identify factors that influence plant invasions. Such comparisons allow us to identify bottlenecks and target future research accordingly. Currently, most studies of plant invasion focus on either the native or introduced range. In addition, there is considerable research effort focussed on single species studies that attempt to identify factors promoting or preventing invasion by determining whether species conform to predictions concerning invasion outcomes. However, my findings in this thesis highlight the importance of understanding ecological processes in both the native and introduced range, while the comparable performance of all three species examined, despite their varying invasion success, underlines the importance of identifying the causes of plant species failure to invade, as well as their success.

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Appendices

Appendix A - *Acacia* species introduced to New Zealand

Table A.1 List of Australian *Acacia* species introduced to New Zealand (Diez *et al.* 2009)

species	synonyms	species	synonyms
Acacia acinacea		*Acacia decora*	
Acacia acuminata		*Acacia decurrens*	
Acacia adsurgens		*Acacia dictyophleba*	
Acacia adunca		*Acacia difformis*	
Acacia albida		*Acacia dodonaeifolia*	
Acacia amblygona		*Acacia doratoxylon*	
Acacia anceps		*Acacia drummondii*	
Acacia ancistrocarpa		*Acacia dunnii*	
Acacia aneura		*Acacia elata*	
Acacia aroma		*Acacia elongata*	
Acacia atramentaria		*Acacia ensifolia*	
Acacia auriculiformis		*Acacia ericaefolia*	
Acacia axillaris		*Acacia estrophiolata*	
Acacia baileyana		*Acacia falcata*	
Acacia beckleri		*Acacia falciformis*	
Acacia bidentata		*Acacia farnesiana*	
Acacia binervata		*Acacia filicifolia*	
Acacia binervia		*Acacia fimbriata*	
Acacia boormani		*Acacia flexifolia*	
Acacia botrycephala	*terminalis*	*Acacia floribunda*	
Acacia brachybotrya		*Acacia furcatispina*	
Acacia brownii		*Acacia genistifolia*	
Acacia burkittii		*Acacia georginae*	
Acacia buxifolia		*Acacia gerrardi*	
Acacia caffra		*Acacia gladiiformis*	
Acacia calamifolia		*Acacia glandulicarpa*	
Acacia cambagei		*Acacia glaucoptera*	
Acacia cangaiensis		*Acacia gracilifolia*	
Acacia cardiophylla		*Acacia granitica*	
Acacia caven		*Acacia hakeoides*	
Acacia cavenia		*Acacia hamiltoniana*	
Acacia chrysella		*Acacia horrida*	
Acacia clunies-rossiae		*Acacia implexa*	
Acacia cognata		*Acacia irrorata*	
Acacia conferta		*Acacia iteaphylla*	
Acacia cowleana		*Acacia ixiophylla*	
Acacia cultriformis		*Acacia jennerae*	
Acacia cuthbertsoni		*Acacia juncifolia*	
Acacia cyanophylla	*saligna*	*Acacia karroo*	
Acacia cyclops		*Acacia kempeana*	
Acacia dawsoni		*Acacia koa*	
Acacia dealbata		*Acacia lanigera*	
Acacia deanei		*Acacia lasiocalyx*	

species	synonyms	species	synonyms
Acacia lasiocarpa		*Acacia retinodes*	
Acacia leiophylla		*Acacia rhetinocarpa*	
Acacia leprosa		*Acacia riceana*	
Acacia ligulata		*Acacia rigens*	
Acacia linearifolia		*Acacia rossei*	
Acacia lineata		*Acacia* rubida	
Acacia linifolia		*Acacia saliciformis*	
Acacia linophylla		*Acacia salicina*	
Acacia longifolia		*Acacia saligna*	
Acacia longissima		*Acacia schinoides*	
Acacia mabellae		*Acacia semirigida*	
Acacia mearnsii		*Acacia silvestris*	
Acacia melanoxylon		*Acacia sophorae*	
Acacia merinthophora		*Acacia sowdeni*	papyrcarpa
Acacia microcarpa		*Acacia speciosa*	lebbeck
Acacia mitchelli		*Acacia spectabilis*	
Acacia mollissima		*Acacia steedmani*	
Acacia mucronata	*mearnsii*	*Acacia stenophylla*	
Acacia murrayana		*Acacia stricta*	
Acacia myrtifolia		*Acacia suaveolens*	
Acacia notabilis		*Acacia subtilinervis*	
Acacia obliquinervia		*Acacia subulata*	
Acacia obtusifolia		*Acacia terminalis*	
Acacia oshanesii		*Acacia trachyphloia*	
Acacia oswaldi		*Acacia transluscens*	
Acacia oxycedrus		*Acacia trineura*	
Acacia paradoxa		*Acacia triptera*	
Acacia parramattensis		*Acacia triptycha*	
Acacia parvipinnula		*Acacia ulicifolia*	
Acacia pendula		*Acacia umbellata*	
Acacia penninervis		*Acacia undulata*	
Acacia podalyriifolia		*Acacia urophylla*	
Acacia polybotrya		*Acacia verniciflua*	
Acacia praecox		*Acacia verticillata*	
Acacia pravissima		*Acacia vestita*	
Acacia prominens		*Acacia victoriae*	
Acacia pruinocarpa		*Acacia visco*	
Acacia pruinosa		*Acacia wattsiana*	
Acacia pulchella		*Acacia williamsonii*	
Acacia pycnantha		*Acacia xanthophloea*	
Acacia quornensis			
Acacia ramiflora			
Acacia redolens			

Appendix B - Details of the populations used in this thesis

Table B.1 Site names (sites codes in brackets), location, sample size, mean temperature and rainfall, overall population size and brief description of each study population in Australia and New Zealand for each species: *Acacia baileyana*, *Acacia dealbata*, *Acacia pravissima*.

species	site (code)	location	sample size	mean max temp (ºC)	mean annual rainfall (mm)	population size	description
Australia							
A. dealbata	Bushrangers Hill (BR)	36° 1'S / 148°44'E	5	18.1	689	>100	Open woodland / agricultural
	Grabben Gullen (GG)	34°34'S / 149°22'E	5	20.6	653	>100	Open woodland
	Rye Park (RP)	34°26'S / 148°59'E	5	20.6	653	>100	Open woodland / agricultural
	Seven Gates Road (SG)	36° 5'S / 148°51'E	5	18.1	689	>100	Open woodland / agricultural
	Talbingo (TB)	35°34'S / 148°18'E	3	19.6	1175	5 - 10	Roadside planting
			n = 23				
A. baileyana	Jindalee Forest 1 (J1)	34°32'S / 148° 2'E	5	22.1	652	5 - 10	Forest gap
	Jindalee Forest 2 (J2)	34°33'S / 148° 2'E	5	22.1	652	10 - 25	Forest gap
	Temora Road (TR)	34°30'S / 148° 3'E	5	22.1	652	5 - 10	Disturbed roadside
	Cootamundra Corner (CC)	34°33'S / 147°58'E	5	22.1	652	5 - 10	Open forest
			n = 20				
A. pravissima	Roadside (RS)	35°36'S / 148°22'E	5	19.6	1174	10 - 25	Forest gap
	Talbingo (TB)	35°34'S / 148°18'E	5	19.6	1174	5 - 10	Roadside planting
	Yarrangobilly Caves (YC)	35°43'S / 148°29'E	5	19.6	1174	25 - 50	Forest gap/disturbed
	Yarrangobilly Village (YV)	35°38'S / 148°27'E	5	19.6	1174	10 - 25	Disturbed roadside
			n = 20				

Continued on next page…

species	site (code)	location	sample size	mean max temp (ºC)	mean annual rainfall (mm)	population size	description
New Zealand							
A. dealbata	Burnham (BU)	43°38'S / 172°15'E	5	16.7	598	>100	Agricultural margin
	Governor's Bay (GB)	43°38'S / 172°38'E	2	17.5	969	25 - 50	Woodland
	Glen Colwyn (GC)	42°37'S / 173°21'E	5	15.8	1051	>100	Agricultural margin
	Little River (LR)	43°45'S / 172°47'E	5	17.5	969	>100	Woodland/agricultural margin
	River Conway (RC)	42°36'S / 173°25'E	5	15.8	1051	>100	River bed
			n = 22				
A. baileyana	Cheviot (CH)	42°49'S / 173°16'E	3	15.8	721	<5	Ornamental planting
	Chattertons Road (CHRd)	43°30'S / 172°25'E	3	16.9	594	<5	Private garden
	Jocks (JO)	43°46'S / 172°47'E	5	17.5	969	5 - 10	Paddock
	McLeans Island (ML)	43°28'S / 172°26'E	5	16.9	594	5 - 10	Trial planting
	SH1 (SH)	43°34'S / 172°25'E	3	16.9	594	<5	Paddock
			n = 19				
A. pravissima	B. Deans (BD)	42°56'S / 173°8'E	4	1538	721	<5	Roadside planting
	Cheviot (CH)	42°49'S / 173°16'E	3	15.8	721	<5	Ornamental planting
	McLeans Island (ML)	43°28'S / 172°26'E	5	16.9	594	5 – 10	Trial planting
	SH1 (SH)	43°34'S / 172°25'E	5	16.9	594	5 - 10	Paddock
	Cashmere Road (RD)*	43°35'S / 172°35'E	3	16.9	594	5 - 10	Paddock/roadside edge
			n = 20				

* Site only used for estimates of pods per inflorescence

Appendix C - Pods per inflorescence - per site

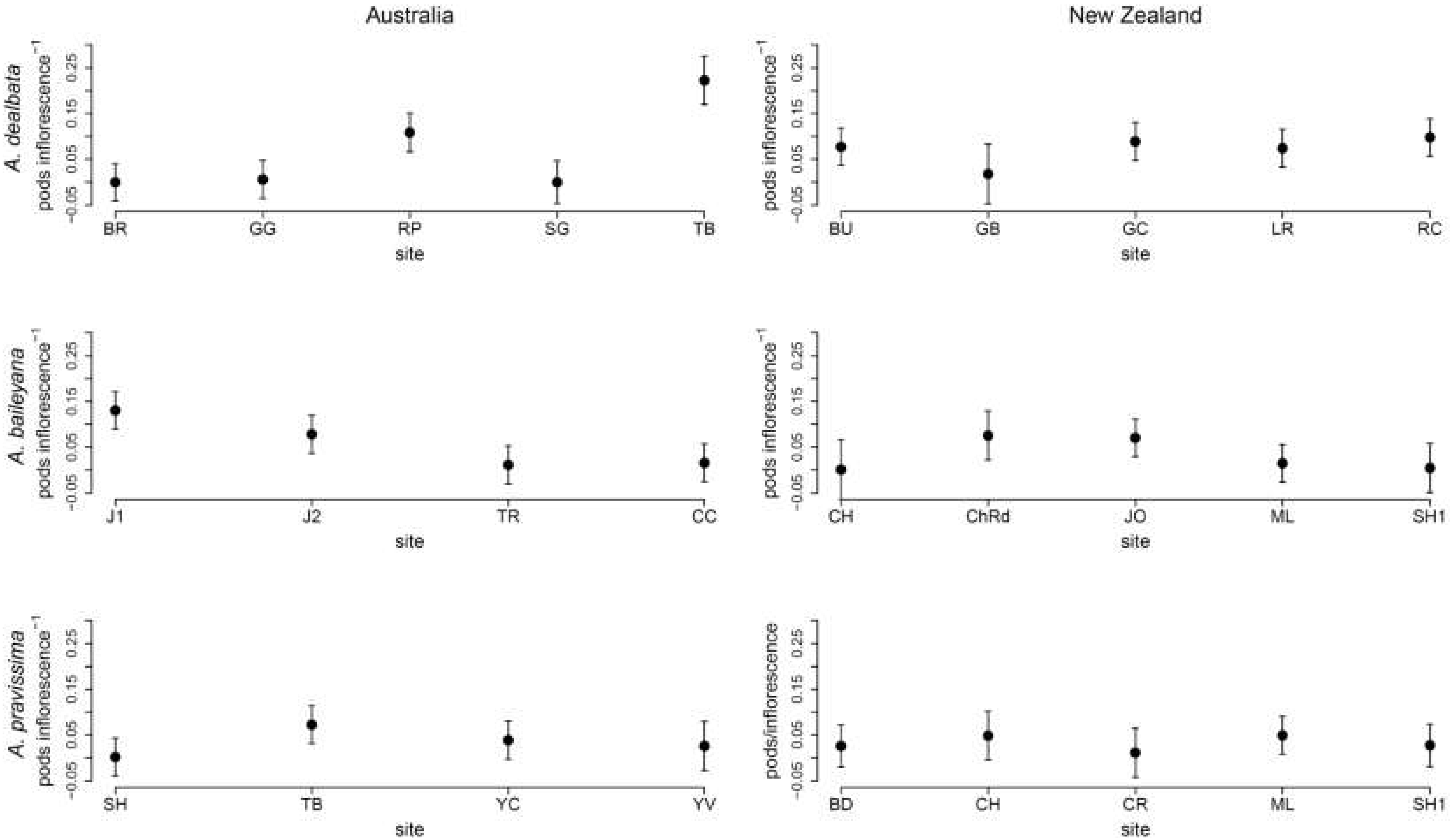

Figure C.1 Pods per inflorescence for the three species of *Acacia* used in this study at each site (Appendix B) in New Zealand and Australia. Bars represent 95% confidence intervals.

Appendix D - Seed fate - per site

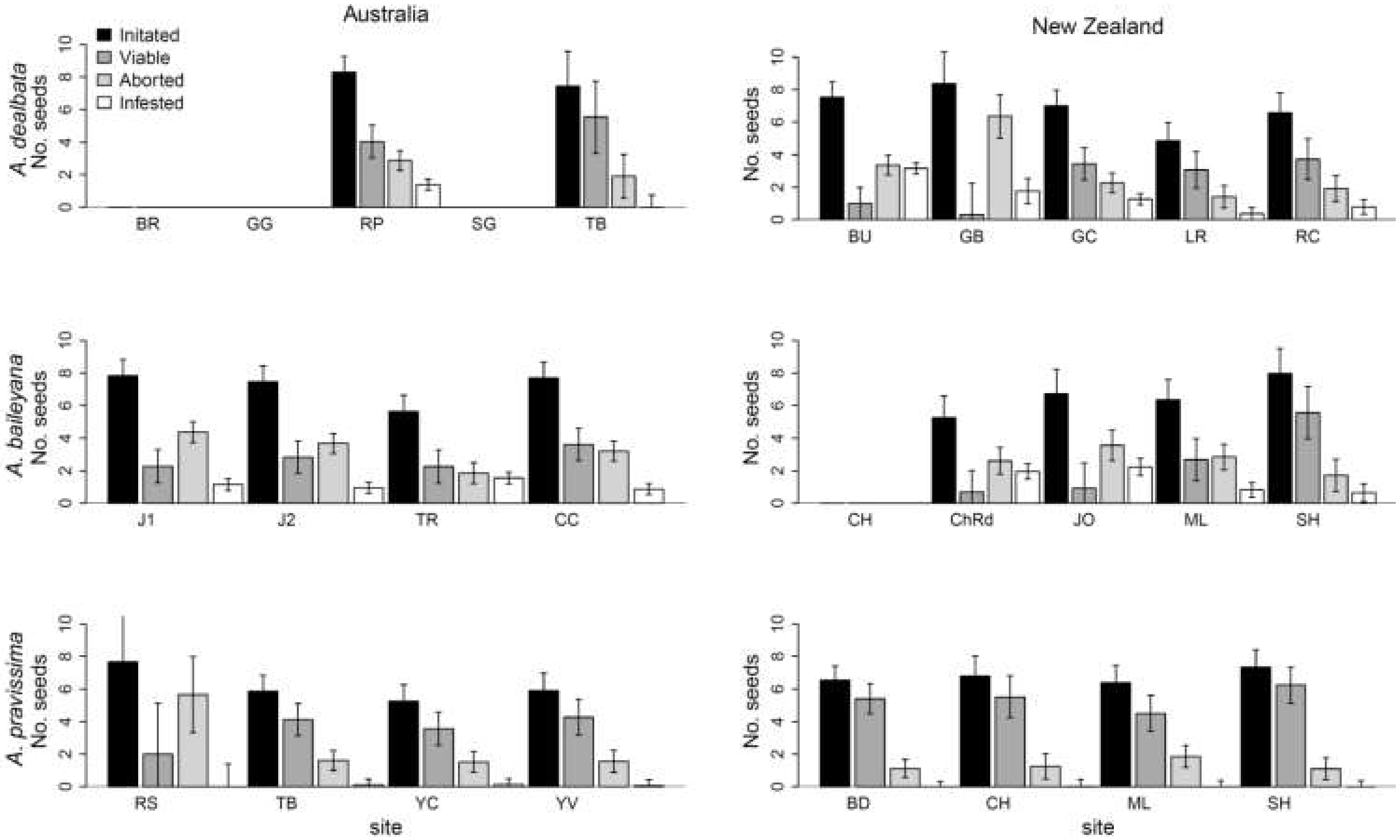

Figure D.1 Number of seeds initiated, total number of viable seeds, number aborted and number infested with insects at each site for the three species of *Acacia* in this study in New Zealand and Australia. Bars represent 95% confidence intervals.

Appendix E - Mean basal diameter for each species in each country

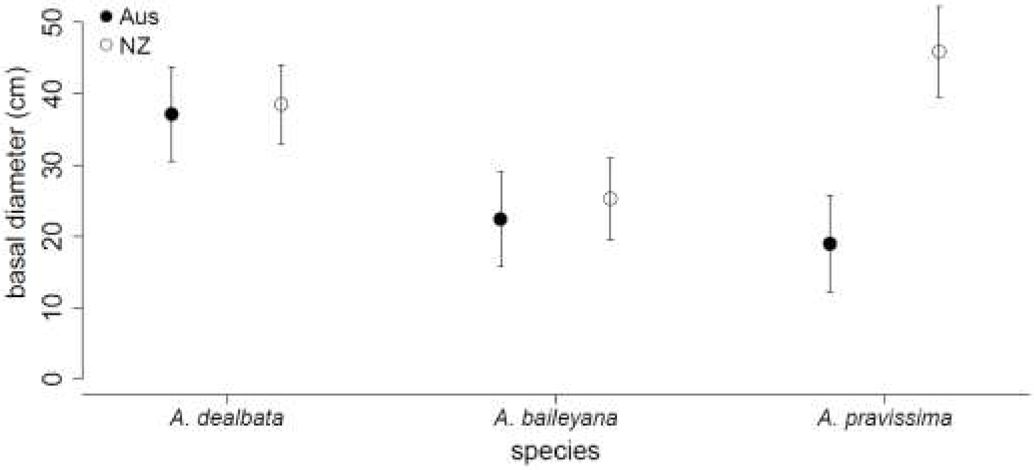

Figure E.1 Tree basal diameter, as an indicator of tree size, for the three *Acacia* species used in this study in their native Australia and introduced New Zealand range.

Appendix F – Seed rain - per site

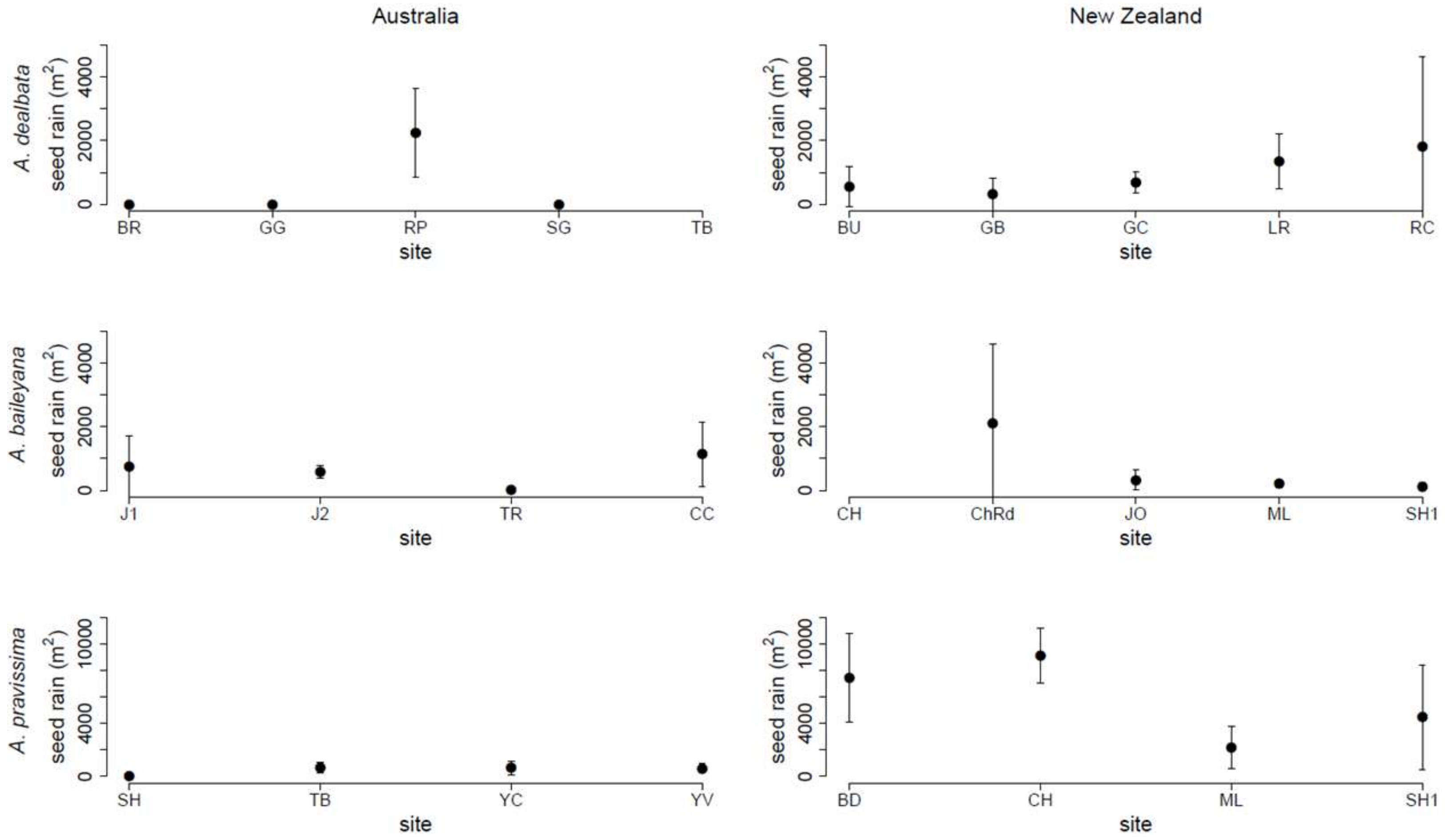

Figure F.1 Seed rain per site for the three species of *Acacia* in this study in New Zealand and Australia. Bars represent 95% confidence intervals.

Appendix G – Seed rain for each species in New Zealand in 2009-2010

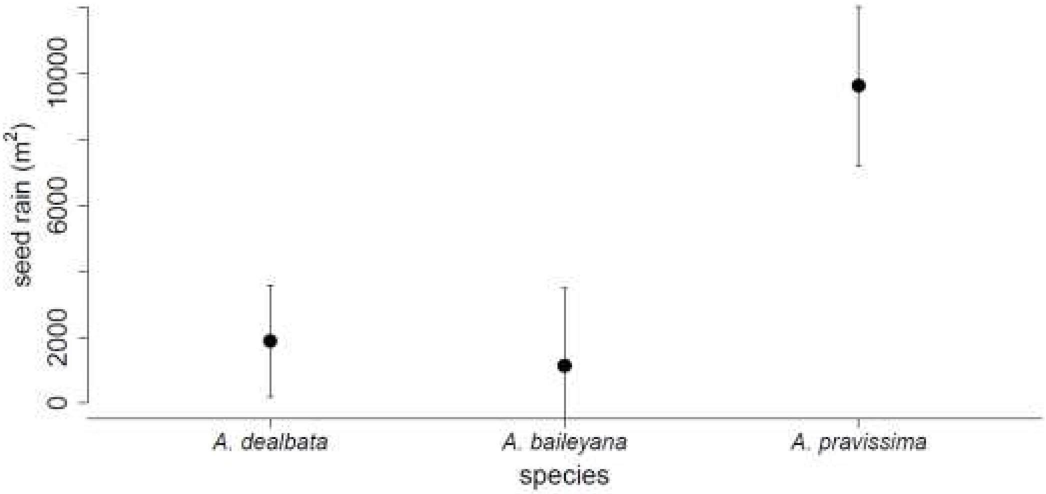

Figure G.1 Seed rain recorded for the three *Acacia* species used in this study in New Zealand in 2010. The data cannot be directly compared as the seed traps were out for variable periods of time, sample sizes were smaller and different sites were used between the two years. However, the general trend indicates that the high seed output recorded for *A. pravissima* in 2011, relative to the other two species, is likely consistent between years.

Appendix H - Details of the model fitted to the data to estimate the overall probability of a diaspore being removed by either vertebrates or invertebrates under each distance and density combination.

We used a binomial distribution that included terms to model both variation among trees, which were nested within sites, and to account for overdispersion:

$$r_{ijk} \sim \mathrm{Binomial}\left(p_{ijk}, n_{ijk}\right)$$

$$\mathrm{logit}\left(p_{ijk}\right) = a + X_{ijk}\beta + t_{jk} + \varepsilon_{ijk}$$

$$t_{jk} \sim Normal\left(s_k, \sigma_t^2\right)$$

$$s_k \sim Normal\left(0, \sigma_s^2\right)$$

$$\varepsilon_{ijk} \sim Normal\left(0, \sigma^2\right)$$

Where r_{ijk} is the number of diaspores removed from the i^{th} dish located under the j^{th} tree at the kth site, p_{ijk} is the probability of diaspore removal for that dish and n_{ijk} is the number of diaspores available for removal (either 1 or 20). The probability of diaspore removal for each dish (on the logit scale) was modelled as a function of an intercept term, a, treatment variables, X_{ijk}, with associated regression coefficients, β, that estimate the effect of each treatment on the response, a term to model differences in removal probabilities among trees within sites, t_{jk}, and an overdispersion term ε_{ijk}, which measures the degree to which diaspore removal probabilities for individual dishes deviate from that expected under a standard binomial distribution conditional on the treatment and tree effects. The individual tree terms were nested within sites and were treated as random effects by modelling them as drawn from a normal distribution with a different mean for each site and variance estimated from the data. Both the site means and overdispersion terms were modelled as drawn from normal distributions with mean zero and variances estimated from the data. We used this model to estimate removal probabilities in the high density treatment when dishes contained 20 diaspores ($n_{ijk} = 20$). Inclusion of the overdispersion term was not necessary in the low density treatment when trays contained one diaspore because such binary (0, 1) response data cannot exhibit overdispersion. Here, the probability of encounter was equivalent to the probability of removal.

To overcome the issue of statistical separation at low diaspore densities (where all or no seeds were taken in a given treatment) and to deal with the hierarchical structure of our experimental design we fitted our models in a Bayesian framework. This allowed us to deal with problems of separation by specifying weakly informative prior distributions for the regression coefficients, which ensured that the data still drove parameter estimation but constrained the coefficients (on the logit scale) within reasonable bounds when the data indicated probabilities of zero or one. Following Gelman (2008) we specified priors for the treatment regression coefficients as coming from a Student-t7 distribution centred on zero with a scale of 2.5, and for the intercept term with a scale of 10. For the tree, site and overdispersion terms we specified non-informative uniform priors (0-100) on the standard deviations following Gelman (2006).

Appendix I - Methods and results figure for a study that investigated invertebrate diaspore preference in New Zealand independently of population size for the three *Acacia* species used in the main experiment.

Methods

We selected up to five trees in up to five populations per species in New Zealand (Table I.1). Where possible, study populations were the same as those used in the main experiment. Where this was not possible, we identified the nearest available population.

We used a similar approach to the main experiment and presented diaspores in Petri dishes modified to allow access to invertebrates only. We presented 20 diaspores with their elaiosome intact, which meant that the data were comparable to the plus elaiosome, high density treatment of the main experiment, and included both distance treatment used in the main experiment (base and 10m).

Diaspores were presented sequentially at each individual tree. The sequence of diaspore species was randomly assigned to each tree such that on the first visit one of three possible species was randomly assigned at each individual, on the second visit one of the two species remaining for that individual was randomly assigned and on the third visit the final species not yet presented at that individual was given. In each instance diaspores were left out for four days before the number remaining was recorded.

To coincide with seed fall for each species and peak ant activity in New Zealand, as well as ensure comparability between this and the main experiment as far as possible, the first two visits in the sequence coincided with the January round of the main experiment in New Zealand, while the third visit coincided with the February round.

Table I.1 Location and sample size for each study population. * indicates populations not used in the main experiment.

Species	Site	Location	Sample size
A. dealbata	Burnham	43°38'S / 172°15'E	5
	Coringa*	43°28'S / 172°26'E	2
	Little River_L	43°45'S / 172°47'E	5
	Glen Colwyn	42°37'S / 173°21'E	5
	Orton Bradley	43°39'S / 172°42'E	2
			n = 19
A. baileyana	Coringa	43°28'S / 172°26'E	5
	Little River_B	43°46'S / 172°47'E	5
	Orana*	43°28'S / 172°27'E	5
			n = 15
A. pravissima	Orana*	43°28'S / 172°27'E	5
	Chattertons Road*	443°30'S / 172°25'E	1
	McLeans Island	43°28'S / 172°26'E	5
	SH1	43°34'S / 172°25'E	2
			n = 13

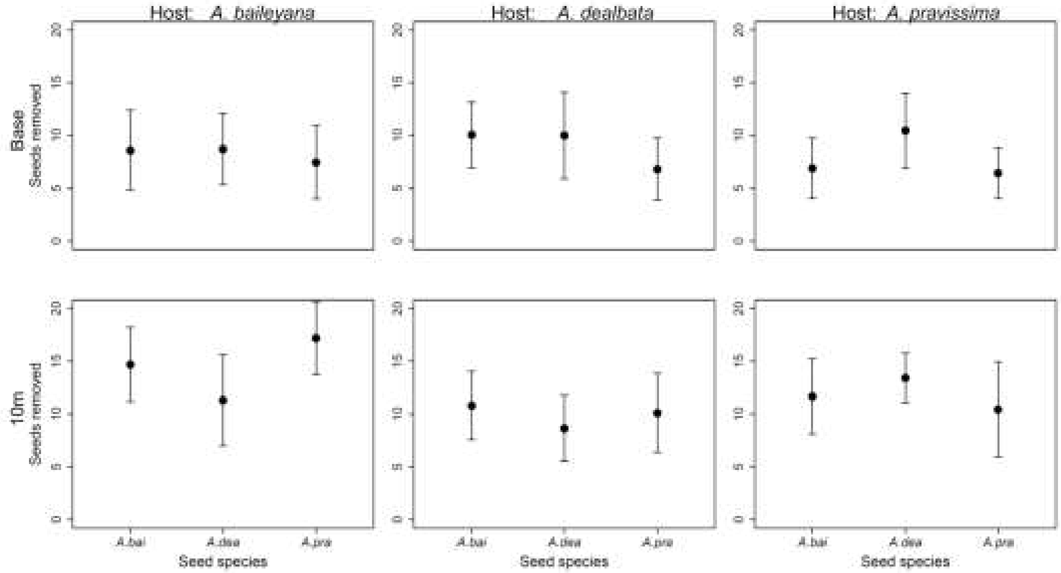

Figure I.1 Mean number of diaspores of each species of *Acacia* removed by invertebrates from dishes at two distances from adult plants of each species of *Acacia* (Host). The data presented include only removals from dishes once they had been encountered. Bars represent standard errors.