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Invasion Ecology and Biology of Blackberries of the Rubus fruticosus *L. agg. in Australia*

John Marshall, B. A. (Hons.) Biol.

School of Biological Sciences, Faculty of Science and Engineering,
Flinders University, Adelaide, South Australia

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Thesis abstract

Background Finding solutions to the negative impacts caused by weeds in natural systems is a very challenging task for researchers and land managers across the world. Many studies of weed taxa and weed management programmes are often hampered by a lack of knowledge of the basic biology and ecology of these taxa and of the processes and mechanisms contributing to the success of invaders in new habitats. A detailed understanding of the competitive and adaptive attributes of weeds, and the role they play in adaptation to new environments and environmental extremes, remains elusive and incomplete for most species.

In Australia, invasive blackberries of the *Rubus fruticosus* L. aggregate (approximately 15 species, most of which are putatively polyploid) are problematic weeds. Surprisingly, little is known about the biological and ecological attributes of individual species within the aggregate. Control and management programmes are typically structured around the aggregate as a whole and have evolved primarily towards finding solutions to the agricultural threat they pose, often at the expense of testing and expanding our understanding of the complex biology and ecology of blackberries and of the factors that facilitate their invasiveness.

Location Southern Australia and South Australia.

Objectives My research identified and investigated the biological and ecological attributes of individual species of blackberries and their potential for range expansion in southern Australia. I estimated the bioclimatic niches of selected *Rubus* taxa and expand the understanding of some of the biotic and abiotic preferences of these species and their relative ability to invade native vegetation, which helps to explain their invasiveness in Australia. I also examined the competitive impacts of three blackberry species on selected native species.

Methods I used five types of species distribution models (SDM), (in the *Dismo* package of R) and constructed predictive maps of the potential ranges of different species within the *R. fruticosus* agg. in southern Australia and in South Australia, based upon known locations of different species of blackberries combined with a set of bioclimatic variables. I then measured the overlaps and similarities of the climatic niches of the different species using environmental niche modelling techniques (ENM) and tested (using two measures of niche similarity) the statistical significance of observed

levels of niche similarity in South Australia, relative to a null hypothesis of random overlap.

In a separate field survey, areas of native vegetation in the Mt. Lofty Ranges region were surveyed for the presence/absence of ten blackberry taxa. I identified five common species that invade native vegetation and developed explanatory generalised linear mixed models (GLMM) to predict the occurrence of the five species based upon abiotic and biotic variables measured at the random field quadrats.

I also measured the growth response of two Australian natives (*Leptospermum continentale* and *Acacia provincialis*) in competition experiments under shade-house conditions and in the field, in which the native plants were planted along with three common blackberry species that invade native vegetation (*Rubus anglocandicans*, *Rubus erythrops*, and *Rubus leucostachys*).

I used flow cytometry methods (FCM) and chromosome counts to assess ploidy level and nuclear DNA content for six blackberry species in their invasive range in Australia using material collected in the Mt. Lofty Ranges. Species in the *Rubus fruticosus* L. aggregate that occur in Australia have been considered likely to be facultatively apomictic and tetraploid with the exception of one diploid taxon.

Findings

Species distribution modelling The maps generated for the blackberry species demonstrated significant variability among the four most common species with respect to their potential invasive ranges. *Rubus anglocandicans* for instance, currently exists over a broad area of southern Australia and has the potential to expand its range primarily at the margins of known populations in all southern states. In contrast, *Rubus erythrops* has a more limited actual range in Victoria, South Australia and Tasmania but my modelling indicates a capacity to expand its range into new areas in Western Australia and New South Wales. Other less common species also display a similar potential.

Environmental niche modelling The climatic niches of seven blackberry species were compared in pair-wise tests using the Hellinger's I_{mod} and Schoener's D statistics by the identity tests and background similarity tests in the ENMTools software package. Niche overlaps were typically less than expected under a null hypothesis of random overlap, demonstrating the presence of niche differentiation with respect to the climatic variables measured. The niche of *R. anglocandicans* was more distinctive than those of the other species of the *R. fruticosus* agg. examined.

Survey of native vegetation Logistic regression analyses of relationships between measured attributes of random survey sites in native vegetation and the occurrence of taxa of the *R. fruticosus* agg.*,* demonstrated that the presence of different species of blackberries varied as a function of different combinations of variables characteristic of each modelled species. For instance, *R. anglocandicans* occurs in habitats characterised by a broad range of aridity but avoids areas of high sunlight intensity in gaps in native vegetation. The presence of *R. erythrops* appeared partly dependent on gaps in native vegetation, whereas the second most common species *R. leucostachys* showed a very low association with native vegetation appearing to prefer unshaded habitats.

Competition Growth responses of two native plant species - *L. continentale* and *A. provincialis* – were measured when grown under shade house conditions in competition with three blackberry treatments (*Rubus anglocandicans*, *Rubus erythrops* and *Rubus leucostachys*). The results showed highly significant effects on growth of *L. continentale* (for above-ground and below-ground weights of plants (both wet and dry weights with the exception of root dry weight), height increase and stem area increase), after a four-month growth period. Growth responses of *A. provincialis* (considered to be a nitrogen fixer) under shade house conditions showed no significant growth effects of plantings with three blackberry species measured over the same period for the same growth parameters. The overall pattern of effects among blackberry species on the growth of *L. continentale* was consistent over all six metrics measured, showing *R. anglocandicans* has the strongest effect and *R. leucostachys* the weakest on growth of this native species.

Chromosome numbers and flow cytometry Estimated chromosome numbers are reported for six blackberry taxa in their invasive range in Australia using material collected in the Mt. Lofty Ranges. Chromosome counts for five taxa (2n=28) are indicative of tetraploidy and chromosome counts for one taxon (2n=14) are indicative of diploidy. Genome sizes estimated using flow cytometry for the tested group of blackberry species were small (e.g. less than 10 picograms), standardised against *Pisum sativum* L. The evidence from chromosome counts did not provide any evidence that hybridisation is occurring between the related diploid and tetraploid taxa examined as no triploid cytotypes were found.

Main Conclusions The maps displaying the potential for range expansion by individual species of blackberries showed several blackberries have apparently not yet reached the limit of their ranges in southern Australia. The multiple model approach combined the power of several algorithms to create a suite of comparative predictive distribution maps of individual blackberry species in southern Australia and in South Australia. Predictions of range expansion at the smaller-scale extent of South Australia predicted potential new range expansions and expansions at the margins of their current distribution for the species examined.

Employing environmental niche modelling (ENM), this study also revealed measurable niche differences that are characteristic of individual species. ENM showed some species were significantly differentiated in terms of their bio-climatic niches, despite the presence of considerable range overlaps.

The results from the ecological survey of the occurrence of blackberries in native vegetation showed individual species displayed a variable tendency to invade native vegetation habitats predicted by a varying suite of abiotic and biotic variables. Results of the glasshouse competition experiment suggest that native plant species that are able to fix atmospheric nitrogen may be more able to compete against some blackberry taxa than species that are not nitrogen fixers. Further field trials are necessary to investigate this possibility.

The survey of variation in chromosome number and DNA content of selected blackberry species in the *Rubus fruticosus* agg. from the Mt. Lofty Ranges indicated that the majority are tetraploid and one is diploid. Further surveys would be interesting to examine the possibility of hybridization among species and to test for further variation in ploidy level.

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Declaration

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

John Marshall, August 2015

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Overview of thesis content

The thesis presents original work examining the biology and ecology of individual species of *Rubus fruticosus* L. aggregate taxa in the Australian context.

Supervisors for the project: Assoc. Profs. Molly A. Whalen, Duncan A. Mackay and Dr. Simon N. Benger.

Research Chapters (Chapters 2-6) have been arranged for easy formatting for publishing as scientific papers (*Diversity and Distributions* and *Annals of Botany*). For this reason, each chapter has a separate reference list. Relevant tables and figures for each chapter are included within the text of the chapter. Supplementary data are at the end of each chapter and listed as appendices.

A table of contents has been included for easy navigation within the thesis.

I wrote all chapters. My supervisors, Duncan Mackay and Molly Whalen are co-authors for the reason of their significant advice and input into all chapters. Simon Benger is a co-author for his significant advice in Chapter 2.

The research chapters are listed below:

Chapter 2 Distribution modelling of species of blackberry in Australia

Chapter 3 Testing niche differentiation among species of the *Rubus fruticosus* L. aggregate in South Australia

Chapter 4 Modelling the biotic and abiotic preferences of blackberry invading native vegetation in the Mt. Lofty Ranges

Chapter 5 Competitive effects of invasive blackberries on selected native Australian plant species

Chapter 6 Chromosome numbers and genome size of the weedy *Rubus fruticosus* L. aggregate in Australia

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Chapter 1

General introduction

The research presented here seeks to expand the biological and ecological knowledge of individual species of the *R. fruticosus* agg., one of Australia's most problematic weeds. Blackberries as a group are included in the Weeds of National Significance (WONS) and are targeted by land managers to contain and limit their spread in southern and eastern Australia (Department of Agriculture Fisheries and Forestry, 2012).

In this introduction, I draw upon general examples in invasive species research and upon studies of invasive *R. fruticosus* agg. species in other invaded ranges, where available, to identify knowledge gaps and to more fully understand the invasive potential of blackberries within Australia. I outline some of the relevant literature to my study of invasion ecology (including some relevant hypotheses in invasion ecology) and the role of some traits and attributes (such as a complex breeding systems) that are believed to aid invasive species to become successful invaders. I describe aspects of the known biology and ecology of the *R. fruticosus* agg. in Australia and identify some of the current problems and issues in weed management.

I briefly describe the importance of predicting the potential spread of weedy plant species in understanding the threat invasive species may pose to biodiversity and the role that species distribution modelling (SDM) and environmental niche modelling (ENM) may play in assisting biodiversity management by expanding the knowledge of the biological and ecological attributes of individual invasive species. Finally, I outline the aims and objectives of my thesis.

The Rubus fruticosus *L. aggregate*

Blackberries of the genus *Rubus* are widely distributed throughout their native range that includes the British Isles, North America, Asia, and Europe. Almost all species of the genus are obligate perennial species (Edees *et al.*, 1988). Blackberries are adapted to a wide range of habitats and in particular, are observed as frequent colonisers of disturbed land (Edees *et al.*, 1988; Clark *et al.*, 2007). Approximately fifteen species of European blackberry in the *R. fruticosus* agg. are naturalised in Australia (Evans *et al.* 2007) and this group is the focus of study in this thesis.

Blackberries as invasive weeds in Australia

The history of blackberries in Australia began during the 1800s where British settlers used blackberries in an expanding fruit and jam industry (Evans *et al.*, 2007). A collection of fifteen or so naturalised species of the *R. fruticosus* agg. are categorised as a group as a weed of national significance (WONS) (Thorp & Lynch, 2000). Infestations are found in every State and threaten Australia's bio-security by negatively affecting the biodiversity of natural ecosystems. The major weed infestations of blackberries tend to occur in areas of higher rainfall, i.e. above the 700mm isohyet in large areas in Victoria, New South Wales, South Australia, Western Australia and Tasmania. Blackberries are adaptable to drier climates and may extend their ranges (Amor, 1973; Agriculture & Resource Management Council of Australia & New Zealand & Australian & New Zealand Environment & Conservation Council and Forestry Ministers, 2000; NSW Department of Primary Industries Weed Management Unit, 2009). Blackberries along with other weed taxa in Australia are responsible for major losses to primary production, estimated to cost more than $4 billion annually (Amor *et al.*, 1998; Thorp & Lynch, 2000; Parsons & Cuthbertson, 2001; Department of the Environment and Heritage, 2003; Sinden *et al.*, 2004). As a major weed, the biology and ecology of individual species are surprisingly under-studied in their introduced ranges in Australia (Evans & Weber, 2003). Adding to this lack of knowledge, individual species are not easy to distinguish, which has resulted in biodiversity management systems targeting the aggregate as a whole in Australia and not adapting to achieving regional eradication of some individual species (Evans *et al.*, 2004; Pyšek *et al.*, 2013).

Amor, as the primary earlier researcher studying invasive blackberries in Australia, established the majority of ecological and biological knowledge of *R. fruticosus* agg., mostly from his studies in forestry reserves in Victoria, Australia. This research, however, provides only general knowledge of the Australian taxa as it was based upon identification of only a limited number of taxa following an older taxonomy (Amor, 1973, 1974; Amor & Miles, 1974; Amor *et al.*, 1998).

A major advance in the study of species in the *R. fruticosus* agg. in Australia was a recent taxonomic study (Evans *et al.*, 2007) and development of an interactive taxonomic key (Barker & Barker, 2005).

Management and control of blackberries in Australia

When exotic plants become invasive in introduced ranges, they present land managers with seemingly intractable problems caused by the negative effects on biodiversity, ecological systems and agriculture. Quantifying and assessing the invasive characteristics of weeds and how biodiversity might be affected is often hampered by a poor understanding of the biological attributes of individual taxa and the way in which exotic taxa affect the dynamics of habitats in natural systems. The most serious weeds appear to have biological and ecological characteristics that aid them in becoming invasive following introduction (Richardson & Pyšek, 2006). In Australia, the general thrust of research into weed species has been directed at enhancing the ability of land managers to find solutions to the negative impacts of weed species and that has often been at the expense of hypothesis testing to try to understand the underlying processes and attributes that enable a plant to become invasive (Sindel, 2000; French *et al.*, 2014).

Existing management and control programmes for *R. fruticosus* agg. in Australia, as implemented by land-managers, have been structured as broadly based upon chemical, mechanical and some biological methods of control. The reasons for this are not only linked to the lack of specific biological and ecological knowledge of individual taxa but also to the difficulty in identifying species of the *R. fruticosus* agg. The generic control and management of the *R. fruticosus* agg. has also concentrated primarily on diminishing the economic threat and returning invaded land to production rather than protecting biodiversity (Amor *et al.*, 1998; CSIRO Entomology, 2003; NSW Department of Primary Industries Weed Management Unit, 2009). Research likewise has evolved towards broad scale management solutions and less so toward establishing an understanding of the biological and ecological mechanisms that assist individual species of the *R. fruticosus* agg. to become invasive (Briese, 2004).

Attempts have been made to establish a rust pathogen (*Phragmidium violaceum* (Schultz) G. Winter, (1880)) as a biological control agent that effectively targets individual species of blackberry, but results so far have been mixed. Releases of the rust have been largely ineffectual possibly due to Australia's dry summers and potentially mismatched host specificity as the rust was collected in Northern France and the origins of the Australian taxa are unknown (Piggott *et al.*, 2003; Gomez *et al.*, 2006; Gomez *et al.*, 2008). Rust impacts on populations of blackberries have also been difficult to analyse as very little follow up observations of individual blackberry species have been performed at release sites where the rust appeared to persist for only short periods (pers. comm. Philip Cramond, Bio-Control Officer, Natural Resource Management (NRM) (Mt. Lofty). One particular study by Piggott *et al.* (2003), attempted to predict the impact of rust introductions at localities in Victoria, Australia using spatial mapping models and disease response data of the most common species, *Rubus anglocandicans* A. Newton. They found that the lack of other species-specific ecological data for *R. anglocandicans* and for the other species that occur in Victoria reduced the model's predictive capacity. A more recent survey of the impact of rust infections on *R. anglocandicans* in Western Australia demonstrated a complex suite of other pathogen infections in some areas where *R. anglocandicans* was established, making it difficult to interpret single causes of the dieback of *R. anglocandicans* and the possible additive effect of co-occurring pathogens (Aghighi *et al.*, 2014).

The major issue relevant to the refining of control measures for the *R. fruticosus* agg. in Australia is that the ecological preferences of the ca. 14-15 individual species of the *R. fruticosus* agg. have not been fully established and consequently, development of targeted programmes directed at the most invasive members of the aggregate based upon empirical evidence has not been possible.

Internationally, several studies have been published on individual species of *Rubus* that detail the ecological preferences of several species in the British Isles and Europe but these studies are of relatively limited value with regard to species occurring in Australia (Taylor, 1971, 2005; Gazda *et al.*, 2007; Clark & Jasieniuk, 2012).

The breeding system of blackberries

The majority of the species of the *R. fruticosus* agg. share a complex breeding system. While the objective of this thesis is not a detailed study of variation in the reproductive system within the *R. fruticosus* agg., I believe it is important to outline the potential role that the complex breeding system of blackberries may play in aiding invasion of natural systems.

Bramble species of the *R. fruticosus* agg. are mostly known as agamo-species that means they are microspecies that reproduce facultatively by agamospermy without gamete reduction (Judd *et. al.*, 2002). Most taxa are closely related asexually reproducing biotypes (Edees *et al.*, 1988; Weber, 1999). Sexual taxa within the *R. fruticosus* agg. group do occur and are thought to hybridise; the hybrids acquiring agamospermy. Agamo-species are considered a reproductive anomaly within flowering plants and the *R. fruticosus* as a group contains only a minority of sexual taxa (Nybom, 1995). Studies in Europe show that the *R. fruticosus* agg. has a large number of agamo-species identified by morphology that are assumed to mostly reproduce asexually (known as apomixis) and it seems can rarely hybridise through sexual processes of gamete reduction (Edees *et al.*, 1988). European blackberries are largely genetically isolated from each other and the majority of species of the aggregate that include the Australian species, appear to be polyploid (Nogler, 1984; Evans *et al.*, 2007; Hörandl, 2010).

Polyploidisation, which is common in plants, often occurs in conjunction with apomixis and has been linked with enhanced weediness in plant taxa but the empirical evidence that connects them is limited (Carter, 2000).

Impact of blackberries on native plant communities

In Australia, the vulnerability of high value biodiversity habitats to invasion by individual *Rubus* species that invade native vegetation is relatively unknown. Davies (1998) found a reduction in native plant diversity in plant communities where blackberries are abundant, as well as adverse effects on some rare and vulnerable plant species and communities within the local Mt. Lofty Ranges, South Australia, but further more detailed studies are required to more fully understand their impacts. More importantly, knowledge of the processes and dynamics of invasion of these northern hemisphere taxa in the Australian context is poor, particularly regarding the potential for hybridisation between individual species of the aggregate. The facultative nature of asexual reproduction among apomictic taxa and the presence of one of the diploid species, *Rubus ulmifolius* var. *ulmifolius* among overlapping populations makes the generation of new hybrids possible in the introduced range of the *R. fruticosus* agg. in Australia.

Hypotheses concerning invasion success

The studies of invasion ecology and the processes and attributes of species affecting the distribution and abundance of taxa have been developed from research in community ecology. Central to the understanding of invasion success is the concept of the niche that helps to form hypotheses and predict and understand why invasive taxa are successful or not, in the niche they occupy.

I describe briefly three hypotheses relevant to the research questions in my study: the abiotic constraints invasion hypothesis, the diversity-invasibility hypothesis and the life history strategy hypothesis.

***Hypothesis 1. Abiotic constraints invasion hypothesis* -** this hypothesis predicts extant taxa will not be able to invade a new area that has abiotic conditions outside their physiological tolerance. This hypothesis relies upon the definition of an organism’s fundamental niche (Hutchinson, 1957) and tests whether successful invaders retain elements of their fundamental niche from their native ranges. These elements can be inherent biotic traits of a taxon such as climatic tolerances. In studies contrasting the abiotic and biotic influences that determine a species’ realised niche, abiotic conditions appear to be the most important factors influencing the success of invasive species (Holway *et al.*, 2002; Menke *et al.*, 2007; Vicente *et al.*, 2010).

In invasion ecology studies, species distribution modelling (SDM) models the ranges of species based upon their abiotic traits and is assumed to represent the fundamental niche (the niche that may be occupied without the influence of limiting factors such as competition). It links traditional niche theory in a practical sense, for purposes of understanding the threats posed by invasive species in introduced ranges (Franklin, 2010).

Hypothesis 2. Diversity-invasibility hypothesis **-** Elton's hypothesis (Darwin, 1859; Elton, 1958) states that higher levels of diversity within communities leads to a greater stability and that novel taxa consequently find it difficult to invade more diverse communities. Applying this hypothesis to ecological studies has yielded mixed results and this suggests that a species' ability to occupy a niche is dependent not just on the level of diversity but also on the geographical size of the ecosystems, where larger areas of high diversity are more protected from invasion than smaller ones (Fridley, 2011). Some evidence suggests that the rate of invasion is negatively correlated to the species diversity of competitors within a community but this pattern appears not to be uniform for all ecological systems (Rejmánek & Richardson, 1996).

Hypothesis 3. Life history strategy hypothesis **–** The life history characteristics of weedy taxa suggested by Baker *et al.*, (1965) & Baker, (1974) have been of continuing interest to researchers (Sakai *et al.*, 2001). Baker's hypothesis posits weedy taxa have a suite of common traits that predisposes them to success. While all traits are not evident in all successful invasive species, traits such as the ability to reproduce sexually and asexually, a resistance and adaptation to environmental stress, a superior competitive ability and superior tolerances to environmental heterogeneity, to name a few, are frequently associated with weedy taxa (Baker *et al.*, 1965; Pyšek & Richardson, 2007). Another important attribute of weedy species is a small genome size as found by Bennett (1972) and Bennett *et al.* (1998) in surveys of several thousand plant species. Supporting this assumption in a different way, Knight *et al.* (2005) found strong evidence to suggest plants with large genome size were constrained in the ecological options available to them. More generally, the characteristics and the adaptive nature of weedy taxa that influence invasion success are assumed to be linked to a complex mix of traits acting in concert (Thuiller *et al.*, 2012).

Some factors affecting why some plants are invasive

Complexities of invasion, competition and the niche

Successful invasions of exotic taxa in introduced ranges are assumed to be the result of the inherent superior characteristics of individual plants (Pyšek & Richardson, 2007). Plants occupying a niche display a suite of traits that advantages them to persist in competition in complex communities (Navas & Violle, 2009; Davies, 2011). In highly diverse community complexes, for example, ecological processes appear to act as biotic filters to mediate the competition of individual species and thereby limit the population expansion of any one individual taxon (Stohlgren *et al.*, 1999; Tilman, 1999).

Empirical studies are yet to show extensively, and in a systematic way, the role that the competition-diversity relationship plays in limiting or mediating invasion of extant taxa (Levine *et al.*, 2003; Levine & HilleRisLambers, 2009). In our study of the *R. fruticosus agg.* complex competition effects may be absent in the introduced ranges of taxa in this group within Australia, but very little is known about individual species' preferences and the role competition plays in limiting their impacts in natural systems.

Hybridisation and invasion

Other processes such as hybridisation, with the concurrent development of polyploid hybrids, are suggested to enhance invasiveness in introduced taxa outside of their native ranges (Ellstrand & Schierenbeck, 2000). Hybridisation events generating allopolyploid hybrids in invasive ranges of taxa have rarely been studied or detected but the process remains a potential source of new invasive taxa (Ainouche *et al.*, 2009). Globally, examples of natural hybridisation such as Louisiana irises (Arnold & Bennett, 1993) and of aggressive global hybrids like *Spartina* species (Ainouche *et al.*, 2004) demonstrate the invasive potential of new hybrids in natural systems. The invasion of *Rubus alceifolius* Poir., on La Réunion Island, was attributed to hybridisation between *R. alceifolius,* a bramble with a native range in south-east Asia, and a closely related native species, *Rubus roridus* Lindl. and also demonstrated the ecological consequences of hybridisation (Amsellem *et al.*, 2001). Recent studies also detected putative hybridisation between native and introduced populations of *Rubus*, in the western United States (Clark & Jasieniuk, 2012) and Europe (Šarhanová *et al.*, 2012), although some progeny were not considered to be invasive by the authors.

In Australian populations of blackberries, Evans *et al*. (2007) considered the possibility of interspecific hybridisation. For the majority of *R. fruticosus* agg. taxa in Australia, Evans *et al.* (2007) reported only one DNA phenotype using the M13 marker.

Rubus anglocandicans A. Newton, for instance, the most common species in Australia, was sampled most widely (ca. 75 individuals) using this genetic marker and found to have predominately one DNA phenotype (Evans & Weber, 2003). Other taxa examined were more variable genetically, including *Rubus ulmifolius,* var. *ulmifolius*. *Rubus ulmifolius* is considered the only diploid sexually reproducing species in Australia (Evans *et al.*, 2007). The latter study noted the need for more research on the reproductive biology of weedy European blackberry species in Australia, particularly the morphologically and considerably variable *Rubus leucostachys* Schleich. ex Sm. complex (Evans *et al.*, 2007).

Consequence of hybridisation, polyploidy and genome size

The diversity of the breeding system in plant taxa in introduced ranges appears to be one of the most influential factors in taxa that exhibit weediness (Ward *et al.*, 2008; Ellstrand, 2009). Considerable evidence suggests that genome size in plants is linked to the trait of weediness, where the synergy of small genome size and higher chromosome numbers resulting from polyploidisation, together may help to explain enhanced invasiveness in some taxa in introduced ranges (Bennett *et al.*, 1998; Pandit *et al.*, 2014). Polyploidy occurs/may occur as a duplication of the genome in some hybrid progeny (leading to increased number of chromosomes) following hybridisation between two compatible diploids or polyploid combinations (Adams & Wendel, 2005). The resultant hybrid progeny may undergo genomic rearrangement that in some cases may impart superior evolutionary and competitive fitness enabling these new hybrids to colonise and dominate new habitats (Soltis *et al.*, 2004; Soltis & Soltis, 2009).

In plants, variation in genome size caused by hybridisation events is known to affect phenological, physiological and life-history characteristics and has been identified as a trait in successful weed taxa (Bennett, 1972; Bennett *et al.*, 1998; Leitch & Bennett, 2004; Lavergne *et al.*, 2010).

Chromosome number and genome size in blackberries

Estimates of chromosome number and the genome size of the majority of the species in *Rubus* subgen. *Rubus,* including the taxa that are invasive in Australia, are incomplete, but the knowledge of the status of the Australian populations of these species can give a valuable insight into whether hybrid events have occurred or are occurring within mixed populations of Australian blackberries (Thompson, 1997; Meng & Finn, 2002).

A recent study using flow cytometry methods (FCM) into the reproductive variability of *Rubus* taxa in eastern Europe (in their native range) by Matzk *et al.* (2000), showed a highest variability of reproductive modes was present in tetraploid brambles where some progeny could be sexually derived (Šarhanová *et al.*, 2012). Another important finding of this study was the detection of approximately 17 species or sub-species that had a triploid genome (considered a transition phase to higher ploidy progeny), and which potentially avoids the process of the triploid block and minority cytotype exclusion (Levin, 1975; Köhler *et al.*, 2010). Rare cytotypes (such as triploids) are expected to exist in conjunction with parental types in nature and form part of this mechanism that facilitates the establishment of more stable higher ploidy progeny and potentially more invasive cytotypes both within native and introduced ranges (Matzk *et al.*, 2007; Köhler *et al.*, 2010).

I consider these questions by using FCM and chromosome counts of a selected group of six blackberry taxa that are invasive in Australia to investigate whether there is ploidy level variation and any evidence of hybrid events within introduced populations of invasive blackberries in Australia. Such knowledge is important in understanding the status of mixed populations of blackberries in Australia.

The research presented in this thesis investigates the biological attributes and the ecological behaviour of individual species of the *R. fruticosus* agg., particularly within the Mt. Lofty Ranges, South Australia and Australia. This research expands our knowledge of the invasive behaviour and characteristics of individual blackberry species that invade native vegetation.

Predicting the spatial extent and the niche of invasive species

Predicting the potential extent of species geographic ranges and the niche using species distribution modelling (SDM) or environmental niche modelling is of great interest to land managers and conservation biologists for estimating the potential spread of species. The field has developed rapidly since the original climatic model BIOCLIM (Busby, 1991) and has expanded with a host of predictive methods to estimate the fundamental niche of an organism. The process of SDM is based on several elementary concepts. First, SDM is a static assessment of the potential distribution of a species and does not take into account the effects of migration or dispersal of taxa within a population or the impact of competition within invaded communities (Franklin, 2010). Therefore, knowledge of the ecology and biology of taxa is of paramount importance in constructing robust, believable models useful in environmental management systems. Second, the fast range shifting nature of invasive species such as blackberries means SDM often violates a basic tenant of Hutchinsonian niche theory, that is, species populations are assumed to be in competitive equilibrium in their environment (Hutchinson, 1957; Huston, 1979; Václavík & Meentemeyer, 2012).

The static assessment of a species range is often an important first step in developing more expansive studies that integrate biological and ecological knowledge of invasive taxa and form a more complete understanding of inherent invasion attributes or traits that advantage exotic species in introduced ranges. Environmental niche modelling (ENM), an extension of SDM that has been developed by Warren (2009) and Glor & Warren (2010), offers a method to compare niche attributes of closely related invasive species. ENM measures niche similarities and traits that empirically reflect the degree of niche similarity or differences between individual taxa in new and existing ranges (Townsend Peterson, 2003, 2011).

In Australia, the potential exists for introduced blackberry species to extend their range and estimates of range expansion using SDM and ENM integrated with other ecological studies enable a further assessment of the potential invasiveness of individual species.

Thesis objectives and aims

The research I present here investigates the ecological preferences and biology of individual species of invasive blackberry that have become naturalised in Australia.

The prime overarching objective of the project is to expand the empirical evidence of the invasion potential and preferences of individual species of blackberry threatening native plant communities.

In the thesis, I use an approach using species distribution modelling (SDM) combined with biological and ecological studies of blackberry taxa in the field and in controlled competition experiments to expand the empirical knowledge of each species and to identify gaps in knowledge of these species.

There are several broad aims to this thesis.

Initially, I use species distribution models to investigate whether populations of individual species of *R. fruticosus* agg. are capable of extending their ranges within Australia taking into account a series of bio-climatic variables (Chapter 2). Spatial data from known occurrences of blackberry taxa that have become naturalised within southern Australia and

South Australia were used to establish the potential range of each species over both extents.

I also examine the degree of relative similarity of the niches occupied by blackberry species comparing pairs of selected blackberry species in the introduced range in South Australia using EMNTools (Warren *et al*., 2010) (Chapter 3). The analysis of the characteristics of the niches of paired blackberry species in this chapter details whether the niches of paired blackberry species are similar in a context of niche differentiation that may occur if some species are adapting to local conditions.

Previously, as part of my honours study, I collected 260 botanical vouchers and these were used in conjunction with approximately 380 existing *Rubus* collections available from the State Herbarium of SA in 2008 for mapping the actual locations of seven *Rubus* taxa. The honours study included ANOVA analysis of data constructed from rainfall statistics for South Australia sourced from the Bureau of Meteorology and other GIS layers available from the School of the Environment, Flinders University and the study did not incorporate any species distribution modelling.

During my PhD studies, I relied upon the information from the botanical vouchers collected in my honours study and in addition, I expanded my *Rubus* collection to an approximate total of 320 vouchers. I utilised data from my specimens and from an expanded herbaria database. I used data from approximately 1600 specimens consolidated from the following herbaria, Western Australian Herbarium (PERTH), State Herbarium of South Australia (AD), The National Herbarium of Victoria (MEL), Royal Botanic Gardens & Domain Trust, New South Wales (NSW), Tasmanian Herbarium (HO), Australian National Herbarium (CANB) and Queensland Herbarium (BRI) for species distribution modelling in Chapter 2 and niche overlap modelling in Chapter 3. Variables for species distribution modelling were sourced from WorldClim, CGAIR and the Japan Metrological Agency.

In the following fourth chapter, I describe the factors affecting the presence and absence of individual blackberry taxa using logistic regression models built upon data collected from field surveys of randomly placed quadrats within native vegetation communities in the Mt. Lofty Ranges, South Australia. Field data from these surveys were analysed to identify the factors predicting the occurrence of the dominant blackberry species that invade native vegetation.

In the fifth chapter, I investigate the competitive abilities (if any) of individual species of blackberry against native species and in native vegetation communities of the Mt. Lofty

Ranges (Chapter 5). I used paired planting of selected blackberry and native species, which were analysed to assess the competitive effects of different blackberry species under a controlled shade house conditions and in a field experiment.

The final research chapter (Chapter 6) expands the basic knowledge of the breeding system of individual taxa. I used flow cytometry techniques to estimate the nuclear DNA content and chromosome counts to estimate ploidy level for six selected blackberry species found within the Mt. Lofty Ranges. This not only established the ploidy level of some taxa in the *R. fruticosus* agg. taxa in their invasive range in Australia but also assessed if there was evidence of interspecific hybridisation between these limited number of taxa that could be indicated by the presence of either triploid or high order cytotypes.

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Chapter 2

Distribution modelling of species of blackberry in Australia

[1]John Marshall, [2] Duncan A. Mackay, [3] Molly A. Whalen and [4] Simon N. Benger

[1, 2, 3] Flinders University, School of Biological Sciences, GPO Box 2100, Adelaide, S.A. 5001, Australia.

[4] Flinders University, School of the Environment, GPO Box 2100, Adelaide, S.A. 5001, Australia.

Abstract

Aim Predict the potential for range expansion of blackberry species across two spatial scales using multiple species distribution modelling, and enable a better understanding of the variation in potential range expansion of blackberry taxa.

Location Mt. Lofty Ranges, South Australia, Australia

Methods We assessed the potential invasive distributions of six species of blackberries across southern Australia and South Australia using GIS and five species distribution modelling (SDM) methods, using similar combinations of bio-climatic variables in the *Dismo* package of 'R'.

Results The predictions show the potential environmental niches of almost all species exceed the geographical presence indicated by herbarium records of each species implying most species have not reached the limits of their introduced ranges. Modelling shows significant variability among species with respect to their potential invasive ranges. *Rubus anglocandicans* for instance, exists over a broad area of southern Australia and is likely to expand its range primarily in marginal local areas. In contrast, *Rubus erythrops* has a more limited range in Victoria, South Australia and Tasmania and modelling indicates a capacity to expand its range to areas in Western Australia and New South Wales. *Rubus leucostachys* is likely to occupy considerable new areas across much of southern Australia.

Main Conclusions The separate predictions were informative in identifying potential expansion of population margins and new unoccupied habitat in both extents for all species across Australia. The multiple model approach combines the power of several algorithms that use several important predictor variables for individual species and creates a suite of comparative maps allowing identification of regional and local areas at risk of invasion over southern Australia and South Australia.

Keywords: **blackberry, *Rubus fruticosus* L. aggregate, invasive species, species distribution modelling, multiple models, SDM, South Australia, Weeds of National Significance, WONS**

Introduction

Invasive plant species within Australia have major economic and ecological impacts. Blackberries are one of the major weed taxa that have been included as one of Australia's worst weeds or 'Weeds of National Significance' (WONS). Approximately fifteen species of the *Rubus fruticosus* L aggregate are thought to occur across Australia with the majority of taxa (10) found in South Australia and some states such as Western Australia having only two taxa identified (Davies, 1998; Agriculture & Resource Management Council of Australia & New Zealand & Australian & New Zealand Environment & Conservation Council and Forestry Ministers, 2000; Evans *et al.*, 2007; Department of Agriculture Fisheries and Forestry, 2012).

Invasive **Rubus fruticosus** *L. aggregate in Australia*

One of the major difficulties in working with blackberries of the *R. fruticosus agg.* is that species are not easy to distinguish and are closely related genetically, which has added to the problems of managing these weeds (Evans *et al.*, 2004; Pyšek *et al.*, 2013). The publication of a taxonomic revision (Evans *et al.*, 2007) has helped to establish which species have been introduced into Australia and which traits can be used to distinguish between them.

European blackberries were introduced into Australia during colonial times, and some species have reached a wide distribution in southern Australia, occupying large localised ranges (NSW Department of Primary Industries Weed Management Unit, 2009).

The current and previous management of weedy blackberries has mostly concentrated on the group as a whole without taking into account the considerable variation amongst individual species in ecological and reproductive characteristics that may influence their spatial distribution and invasiveness. Thus, in their introduced ranges in Australia, there is still a limited understanding of the invasion potential and the habitat preferences of individual blackberry species, and fine-scale studies are needed to enhance and refine management programmes where individual species are resistant to current protocols (pers. comm. Philip Cramond, Bio-Control Officer, Natural Resource Management (NRM) Mt. Lofty, Evans & Weber, 2003; Evans *et al.*, 2007).

Invasive species modelling

Species distribution modelling or SDM is often used to relate species distributions to known abiotic environmental variables. It may be used to predict an invasive species' potential range and thus can be used for improving the understanding of invasive species in a framework of management and can also produce valuable information on the environmental correlates of invasiveness of species in new ranges. For example, species distribution modelling of the spatial patterns of invasion has been successfully used to predict the rate and trajectory of invasive species within Europe and North America, information which was then used in conservation management programmes to enhance the success of on-ground control strategies (Roura-Pascual *et al.*, 2009; Elith *et al.*, 2010; Barbosa *et al.*, 2012).

SDM does not always focus on invasive species but can be a valuable tool in assessing a variety of spatial effects in natural systems. For instance, SDM has been used to examine the impacts on native vegetation of inter-specific competition and climate change, providing data for the development of the adaptive management of threatened species in New Zealand (Leathwick, 2001; Leathwick & Austin, 2001). Other applications of SDM include the identification of biodiversity hotspots and locations of vulnerable plant communities, allowing an assessment of the potential impact of climate change and adding valuable knowledge for conservation and management of rare species (Scheldeman & van Zonneveld, 2010; Guerin & Lowe, 2012; O'Donnell *et al.*, 2012).

Modelling of invasive species presents the researcher with a number of difficulties. Some of these difficulties are structural in the way in which SDM is approached and other difficulties arise in the way ecological theory links to SDM (Franklin, 2009). Authors such as Austin (2007) suggest a need for a better conceptual SDM framework that both integrates current ecological theory and standardises the approach when modelling species' potential niches in geographic space.

One of the difficulties in understanding the potential habitat as described by SDM is that it often assumes populations of species are in competitive equilibrium in their environment (Austin, 2007; Franklin, 2009). This is not the case in modelling the potential distribution of 'invasive species', as by definition they are fast range-shifting, highly adaptive and superior competitors (Hutchinson, 1957; Huston, 1979; Václavík & Meentemeyer, 2012). In Australia, blackberries are relatively new introductions and some species may not have reached the full extent of their ranges determined by the bioclimatic constraints and therefore modelling of current distributions is likely to show a potential significant increase in range. In addition, in Australia several species of blackberry are known to have been introduced throughout the 1800s; however, historical records are too fragmentary to allow a precise ranking of the times of individual species introductions or of the identity of the species involved (Giles & Pascoe, 1868).

Another of the constraints in applying SDM to real world scenarios is the lack of transferability of predictor variables between model systems (Franklin, 2010). This is important when examining groups of closely related invasive species in different ranges and is suggested by Thuiller *et al.* (2005) as one of the key ingredients needed to more effectively estimate the potential habitat of invasive taxa in introduced ranges and thereby allow effective SDM of multiple species to be applied to management systems.

Rubus are relatively new invasive species in Australia are of particular interest to land managers as they are a serious threat to biodiversity. They have not reached the potential extent of their introduced ranges in most southern Australian states and the competitive behaviour of individual species is poorly understood. Much weed research within Australia has concentrated on the need to find solutions to the agricultural threat that exotic taxa represent with much less emphasis on hypothesis testing of questions designed to more fully understand the processes of invasion and consequential impacts on biodiversity (French *et al.*, 2014). Some evidence suggests introduced weeds undergo a profound shift in their climatic tolerances when they occupy new areas leading to an under-assessment of their invasion potential, but this aspect remains inadequately investigated (Beaumont *et al.*, 2009). For example, Gallagher *et al.* (2010) found that in a study of 26 minor weed taxa introduced into Australia, the majority of species (19) exhibited a shift in their climatic and biotic range tolerances when matched to their native ranges, but they were unable to link the mechanisms that apparently led to these shifts.

In the Australian context, the basic question of why some exotic plant taxa are both successful in invading native systems and in becoming problematic weeds remains unanswered (French *et al.*, 2014).

Ecological and biological research of individual blackberry in Australia

Previous SDM work in Australia on the biology and ecology of blackberries has generally been limited in scope to individual blackberry taxa and thus has limited application in contributing to adaptive management strategies of the group as a whole. For instance, as part of the continuing theme of finding solutions to the weed problem, Piggott *et al.* (2003) attempted to predict the impact of rust infections specific to blackberries in discrete localities in Victoria, Australia using spatial mapping models and disease response data of a single species *Rubus anglocandicans* (A. Newton). The lack of additional species-specific ecological data for *R. anglocandicans* and for the remaining seven species that occur in Victoria limited the model's interpolation capacity and restricted the predictive capacity to areas that were not suitable for rust. Other studies utilising species distribution modelling (SDM) of blackberries in forestry reserves within Australia have also not accounted for interspecific variation among individual members of the *R. fruticosus* agg. and consequently are of limited value for interpreting habitat variation and preferences of the majority of different blackberry taxa that occur in native systems (Lindenmayer & McCarthy, 2001; Dehaan *et al.*, 2007).

Aims

The major aim of our study is to use species distribution models to gain an understanding of the variation among individual blackberry species in their potential to occupy new areas that are not presently invaded. A second aim of our study is to assess the potential range of selected blackberry species across two spatial scales using species distribution modelling techniques.

Our study results may better inform land managers for conservation planning and management purposes about the threat potential of individual species of this aggregate to spread into areas and regions of southern Australia where they do not presently occur.

Methods

Study species

Blackberries are long-lived perennials and most are facultative pseudogamous apomicts able to generate viable seed without gamete reduction (Clark *et al.*, 2007). A less complex vegetative reproduction is also common through development of underground rhizomes and above-ground primo-canes that apically tip root in the autumn and form new plants in the spring (Amor *et al.*, 1998). Blackberries are adapted to a diversity of habitats in both their native and introduced ranges worldwide, from the Arctic Circle to the tropics (Clark *et al.*, 2007).

We assessed and compared the potential of six species (*Rubus anglocandicans* A. Newton., *Rubus leucostachys* Schleich. ex Sm., *Rubus erythrops* Edees & A. Newton, *Rubus rubritinctus* W.C.R. Watson, *Rubus laciniatus* Willd. and *Rubus ulmifolius* Schott. var. *ulmifolius*) to expand their ranges over both southern Australia and South Australia (Fig. 2.1) using distribution models.

Data and data preparation for SDM

Data on the recorded distributions of blackberry species were sourced and collated from the Western Australian Herbarium (PERTH), State Herbarium of South Australia (AD), The National Herbarium of Victoria (MEL), Royal Botanic Gardens & Domain Trust, New South Wales (NSW), Tasmanian Herbarium (HO), Australian National Herbarium (CANB), Queensland Herbarium (BRI), and the Australian Virtual Herbarium (The Council of Heads of Australasian Herbaria, 2012) (Table 2.1). We also collected new vouchers (approximately 300 individuals) and occurrence data of blackberry species from populations in the Mt. Lofty Ranges at a spatial precision of ± 2.5 metres determined by GPS. These new samples were identified using "The *Rubus* Identification Key" (Barker & Barker, 2005) and confirmed by Dr. David Symon at the State Herbarium of South Australia (AD). Herbarium data were inspected for precision and accuracy in a number of ways. We used a preliminary mapping (GIS) of these data to detect location errors in point data against topographical maps. For example, several herbarium specimens were projected off the coast and data from these specimens were discarded (Newman, 2012).

Table 2.1 Number of georeferenced *Rubus fruticosus* agg. species by herbaria in Australia in cleaned data totals by State

Location	**Sp. 1***	**Sp. 2***	**Sp. 3***	**Sp. 4***	**Sp. 5**	**Sp. 6**	**Sp. 7***	**Sp. 8***	**All *Rubus***
Tasmania	61	12	4	94	0	0	6	0	177
Victoria	169	36	45	147	0	0	0	45	442
Western Australia	189	0	1	0	0	0	0	74	264
South Australia	662	98	124	187	39	33	173	178	1494
New South Wales	298	0	10	44	8	0	0	35	395
A.C.T.	26	0	0	9	0	0	0	3	38
Queensland	37	0	0	0	0	0	0	0	37
Total Southern Australia	1442	146	184	481	48	33	179	335	-
Cleaned data totals									
Southern Australia	642	51	53	174	36	10	79	122	-
South Australia	280	38	53	92	16	10	79	48	

Species codes; Sp.1 *R. anglocandicans*; Sp.2 *R. erythrops*; Sp.3 *R. laciniatus*; Sp.4 *R. leucostachys* Sp.5 *R. phaeocarpus*; Sp.6 *R. riddelsdellii* Rilstone; Sp.7 *R. rubritinctus*; Sp.8 *R. ulmifolius* var. *ulmifolius*. Asterisks * denotes the six species included in the study

We removed records of duplicate herbarium specimens of blackberry species (multiple records of the same collection) but included those that had incomplete spatial referencing but where their spatial location could be determined from other data contained in the specimen's location description. Pre-GPS herbarium specimens were also included when a complete locality description was present.

Species distribution modelling with 'R'

We used the 'R' package 'Dismo', available URL http://cran.r-project.org/web/packages/dismo/index.html (Hijmans *et al.*, 2012), extensively in this study of species distribution modelling of invasive European blackberries in the southern half of Australia and in South Australia.

Multiple model approach

Using a multiple model approach we used several correlative, mechanistic and geographic models to make a range of predictions that were tested and compared against different extents (region x and region y, Fig. 2.1). We used the cleaned data totals of each blackberry species with the two extents of southern Australia and S.A. (Table 2.1).

Within the 'Dismo' package the algorithms used were BIOCLIM (Busby, 1991) (a climatic envelop model), convex hull, a binomial Generalised Linear Model, (GLM) (Guisan *et al.*, 2002), and three correlative models, DOMAIN (Carpenter *et al.*, 1993), Mahalanobis distance (MAHAL) (Mahalanobis, 1936), and MAXENT (available URL http://www.cs.princeton.edu/~schapire/maxent/) (Phillips *et al.*, 2004; Phillips & Schapire, 2006; Phillips & Dudik, 2008; Elith *et al.*, 2011) which is based on maximum entropy.

The outputs of two models (GLM and MAXENT) were compared using 500, 2,000 and 10,000 random background points, confined by the masking function in 'Dismo' to the extents of interest and excluding areas with no data (e.g. marine areas and water bodies) in both extents. After testing, we used 5,000 background points for both regions of the final models as this generated the best area under the curve (AUC) values calculated by using the independent presence-absence data of the six species of the *R. fruticosus* agg. (Table 2.1).

As part of model validation, data partitioning was used to split each dataset into subsets where one was used to train the model and the other subset was used to assess the model's performance (Anguita *et al.*, 2012). We tested various data partitioning with data sets of *R. anglocandicans* and *R. leucostachys* and found data partitions at 50% test and 50% training within MAXENT, set on auto features, generated the best model fit of these two species (Phillips & Dudik, 2008). We then used this data partitioning setting for all other species.

Extents

We used different extents for species distribution modelling; southern Australia (region X, Fig. 2.1), (decimal degrees (dd) latitude -30.000000, -44.008333; dd longitude 112.991667, 154.000000) and a smaller extent of South Australia (region Y, Fig. 2.1), (dd latitude -32.19166, -36.116665; dd longitude 134.283349, 140.508349. The spatial referencing used for all mapping was GCS, Geodetic Datum of Australia (GDA) 1994. The extents (regions X & Y, Fig. 2.1) were chosen with reference to the updated Köppen-Gieger climatic classification, which represents the dominant climatic conditions that exists between 30-40 decimal degrees (dd) south (Peel *et al.*, 2007).

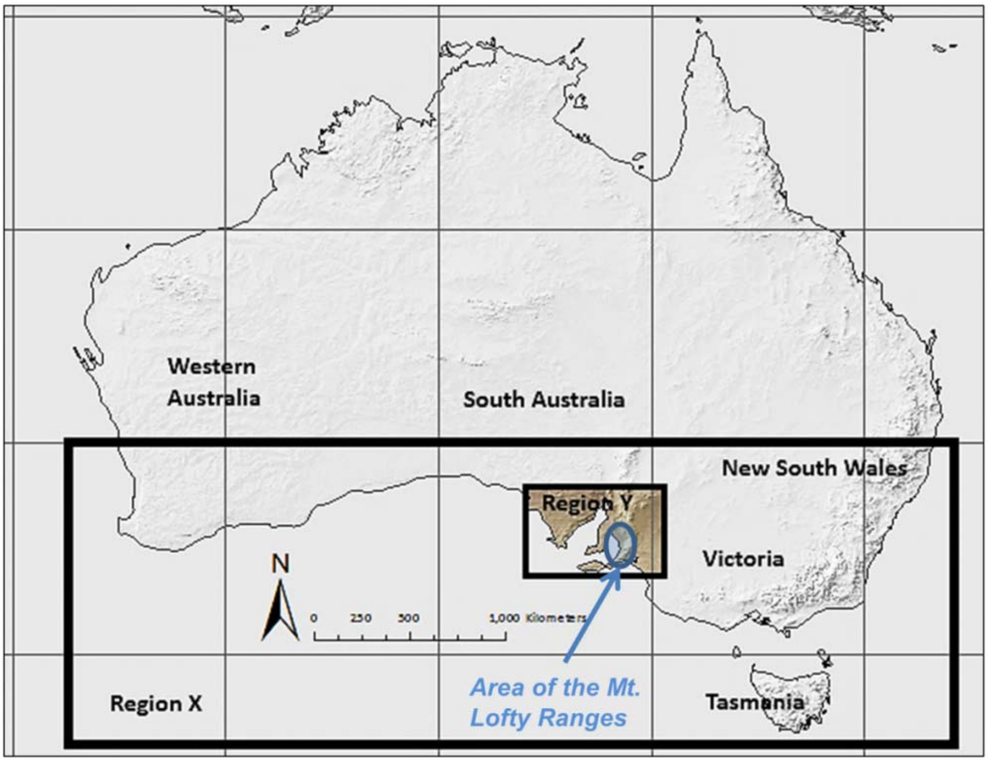

Figure 2.1 Map of Australia with the extents of the predictor layers, of southern Australia (region X) and South Australia (region Y) and the location of the Mt. Lofty Ranges, mapping elements sourced from Patterson and Kelso (2013).

We chose extents to limit the possibility of including extraneous areas where no populations of blackberry are likely to occur, such as the interior arid regions, because

GLM (Guisan *et al.*, 2002) and MAXENT (Phillips & Dudik, 2008; Phillips *et al.*, 2009) models, require both presence and absence data which when drawn from biological and climatically unsuitable regions will over fit the models.

Data on the native home ranges of plant species would also improve the ability of researchers to contrast and compare the bioclimatic preferences of invasive taxa in new ranges. Several studies of invasive species have highlighted the effectiveness and improvement of such information for the outcomes of models in predicting future expansion of invasive ranges (Gallagher *et al.*, 2010; Webber *et al.*, 2011). Even though there is a long history of blackberry studies in Great Britain and Europe (Crane & Darlington, 1927; Heslop-Harrison, 1953; Watson, 1958; Newton, 1980; Edees & Newton, 1988), detailed information on the home ranges of the majority of blackberry species found within Australia in a form suitable for species distribution modelling is not available or accessible.

Predictor layers

We obtained the primary predictor variables from a number of sources, including high-resolution interpolated climate surfaces available from WorldClim.org (http://www.worldclim.org/) (Hijmans *et al.*, 2005), as well as interpolated surfaces of the global aridity (aridity index (AI)) (and global potential evapotranspiration (PET) available from http://www.cgiar-csi.org/ (Zomer *et al.*, 2007; Zomer *et al.*, 2008; Consultative Group on International Agricultural Research (CGIAR) Consortium for Spatial Information, 2012). The global aridity index indicates moisture availability for potential growth of vegetation excluding the effect of soil condition to adsorb and hold water (UNEP (United Nations Environment Programme), 1997). We also used a gridded solar exposure layer from the Australian Bureau of Meteorology. This sunlight layer was derived from satellite imagery, processed from the Geostationary Meteorological Satellite series operated by Japan Meteorological Agency and from GOES-9 data operated by the National Oceanographic & Atmospheric Administration (NOAA) of the Japan Meteorological Agency (Bureau of Meteorology, 2007). We created the masking layers of each extent (which excluded marine areas) for the analyses from data sourced from the Australian Government Department of Sustainability Environment Water Population and Communities (2011). We re-sampled and interpolated the data layers using a variety of GIS software (ESRI® ArcMap®; DIVA-GIS v7.5.0), to a standard 30-second gridded resolution so all layers were spatially consistent for SDM.

The inclusion of soil data, when available, may improve the predictive ability of SDM models for blackberries (Buckley *et al.*, 2010; Coudun *et al.*, 2006); however, complete information on the soil preferences of blackberries is lacking for most of the 2000+ species in both their home and invasive ranges with the exception of a few species in their home ranges not found within Australia (Taylor, 2005; Gazda & Kochmanska-Bednarz, 2010).

Predictor layer – selecting principal layers

We used MAXENT in selecting the predictor variables that contribute most to the modelled outputs for both extents. We ran each group of the primary monthly variables of rainfall, temperature maximum, temperature minimum, temperature mean, solar exposure and PET separately with the aridity index (AI) using the presence only data of *R. anglocandicans* and *R. erythrops* in the two extents (these two species have the largest data sets) (Table 2.1, southern Australia and South Australia.). This resulted in the selection of a subset of eight variables from the original 128 monthly variables (Table 2.2).

Table 2.2 Summary of predictor variables and source

Predictor Variable	Source
Global Aridity Index	CGIAR
Mean PET for December	CGIAR
Mean Solar Exposure for August	Australian Bureau of Meteorology
Mean precipitation for July	WorldClim Global Climate Data
Mean precipitation for December	WorldClim Global Climate Data
Maximum temperature for June	WorldClim Global Climate Data
Mean temperature for January	WorldClim Global Climate Data
Minimum temperature for July	WorldClim Global Climate Data

Results

Southern Australian species distribution modelling

The southern Australian models (GLM, BIOCLIM, DOMAIN Mahalanobis and MAXENT) of the distribution of the blackberry species as a group (Fig 2.2) show blackberries are potentially capable of occupying habitats and areas in addition to known locations but with considerable variability among individual species. Differences in the pattern and extent of potential new ranges in models for each species demonstrated that the less common species are likely to occupy considerably larger new ranges relative to their current ranges than the most widely distributed species *R. anglocandicans*. Models of *R. anglocandicans* suggested a less extensive range expansion, confined to the margins of the current distribution and regions adjacent to areas of known occurrences, when compared with the other taxa (Fig 2.2 (a-d) App. Figs. A2.1-A2.12).

For several species, for example *R. leucostachys, R. erythrops*, and *R. rubritinctus*, there are large areas of southern Australia (Fig. 2.2) that are climatically suitable, particularly in Western Australia, where at present no known collections of *R. leucostachys* have been recorded (AD) (Fig. 2.2 (a)). All five modelling algorithms used in this study predicted that the south-west corner of Western Australia was climatically suitable for this species. Other potential habitat across southern Australia that is climatically suitable for this species and where it is presently absent were also identified in South Australia, Victoria, Tasmania and New South Wales (Fig. 2.2 (a)). The other two species modelled using multiple algorithms, *R. rubritinctus* (Fig. 2.2(b)) and *R. erythrops* (Fig. 2.2 (c)), show a comparatively greater potential spread relative to current ranges into new areas than *R. anglocandicans*. The predictions of areas that are climatically suitable for these species suggest that areas of south-western Western Australia and southern Victoria are at risk of being occupied by these species.

For the remainder of the species in the aggregate, including *R. ulmifolius* and *R. laciniatus*, predicted ranges were variable but also indicate that these species have not reached the limit of their potential ranges (App. Figs. A2.9-A2.12).

South Australian species distribution modelling

Species distribution models of blackberries in South Australia show most models (GLM, BIOCLIM, DOMAIN Mahalanobis and MAXENT) predicted at a smaller scale than the larger southern Australian extent allowing better fine-scale resolution of predicted margins of new areas of potential infestation (Fig. 2.3). The modelling of *R. leucostachys* (Fig. 2.3) implies this species might occupy some of the drier parts of South Australia on the Eyre Peninsula and in the interior (Fig. 2.3). Comparing this result to the modelling across southern Australia (Fig. 2.3) predicted large areas of potential habitat in the drier interior parts in most states and particularly South Australia.

The finer scale modelling, when compared to the large-scale model outputs for southern Australia, demonstrate that the predictor variables used in this study are generally consistent with the prediction generated for southern Australia. Resolution though of margins of predicted habitat for all blackberry species was at a finer scale in South Australia and in particular, model outputs of *R. anglocandicans* (Fig. 2.3) revealed potential unoccupied habitats exist on the Yorke Peninsula and Kangaroo Island (Fig. 2.3). Models using the bioclimatic envelope model (BIOCLIM) and the point distance model (DOMAIN) generated similar maps representing a more limited range expansion for *R. rubritinctus* and *R. erythrops*, which currently have quite restricted ranges (Fig. 2.3). For the remaining two species, *R. ulmifolius* and *R. laciniatus,* variation between the model outputs is considerable with predicted new areas for *R. ulmifolius* the most extensive in areas of the Yorke Peninsula (App. Figs. A2.19-A2.20).

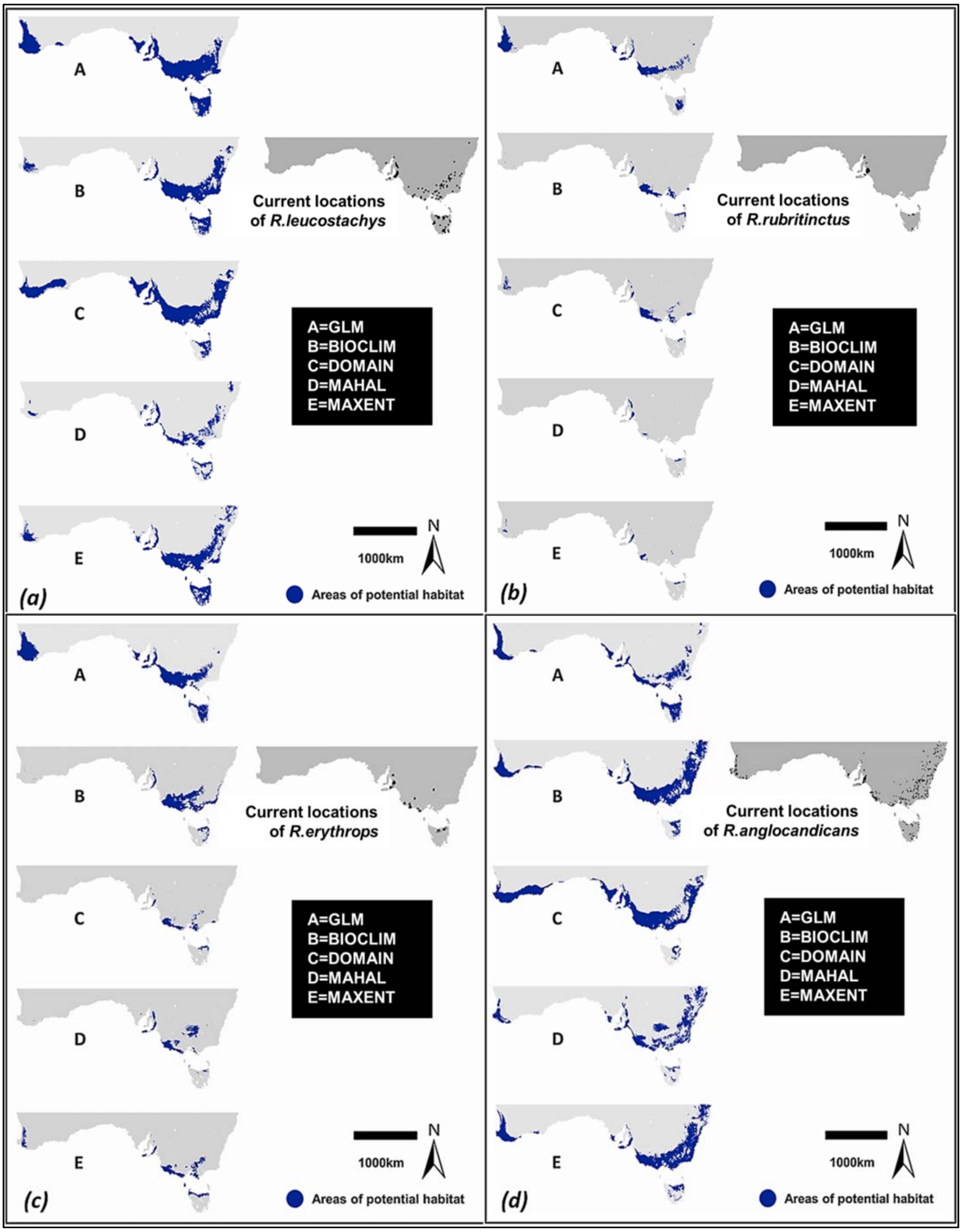

Figure 2.2 Summary maps of potential habitat for four *Rubus* species estimated with five SDM models (left side in each box) compared to current locations (right side in each box) in Southern Australia Note: *a=R. leucostachys, b=R. rubritinctus, c=R. erythrops, d=R. anglocandicans*

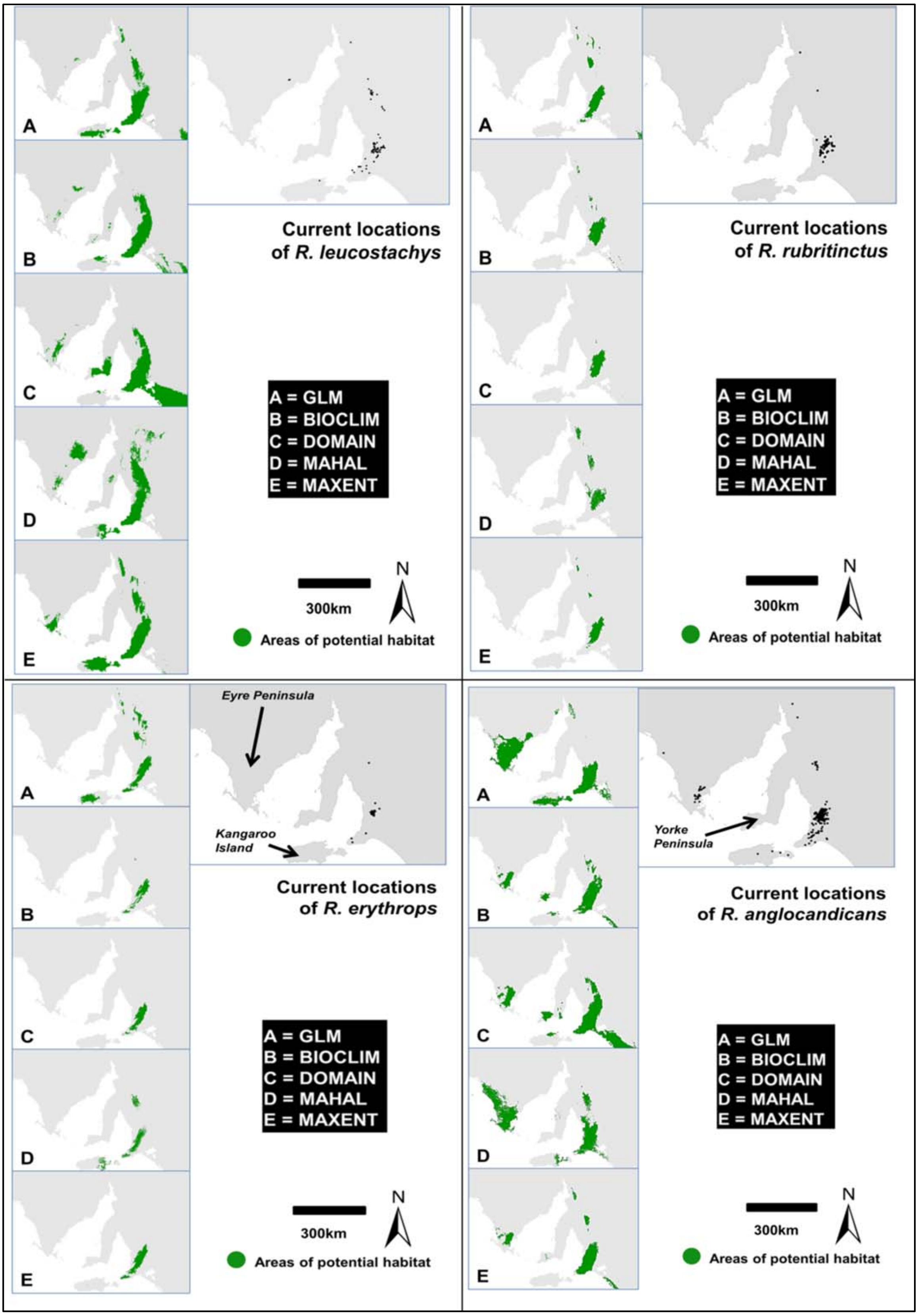

Figure 2.3 Summary maps of potential habitat for four *Rubus* species estimated with five SDM models (left side in each box) compared to current locations (right side in each box) in South Australia

Discussion

The species distribution modelling of individual species of the *R. fruticosus* agg. based upon underlying bio-climatic information, demonstrated a potential for expansion of all species into climatically suitable habitats across considerable areas of southern Australia, both regionally and locally. Predictions over two differing scales provided a better understanding of the invasion potential of individual species of blackberries in Australia at different resolutions. Each blackberry species displayed a unique set of ecological and habitat preferences and consequently a different potential to extend their invasive range in the landscape. The former are further explored in Chapter 3.

The predictions of some models show the potential environmental niches of almost all species exceed their geographical range as indicated by herbarium records of each species for the algorithms employed, implying most species have not reached the limits of their introduced ranges.

While the most widely distributed blackberry species across southern Australia, *R. anglocandicans*, may extend its populations only in local areas and at the margins of known populations, the less common species such as *R. erythrops* and *R. rubritinctus* show even by the most conservative model estimates (MAXENT), that they have the potential to greatly expand their ranges into new areas in all southern Australian states.

Models indicted Western Australia may be at particular risk to invasion by the second most common species *R. leucostachys*, which is only found in the eastern states.

Variation amongst models

In our comparative SDM framework, variation amongst all models (GLM, BIOCLIM, DOMAIN Mahalanobis and MAXENT) appeared a function of the habitat preferences represented by combinations of predictor variables specific for each blackberry species. Some groups of model outputs for individual species were consistent in identifying the region at risk within the study extent, albeit the region area’s shape and margins are different. A good example is *R. leucostachys* modelled in South Australia (Fig 2.2 & App. Figs. A2.15-A2.16) where GLM, BIOCLIM, DOMAIN and Mahalanobis identified potential new local areas with greater margin definition and at a finer scale of detail than for *R. leucostachys* in southern Australia (Fig. 2.3 & Figs. App. A2.4-A2.5).

Overall, the predictions for the South Australian extent in Figure 2.3 were more informative in identifying finer grain expansion of margins of potentially new habitat locally for *R. anglocandicans*, *R. leucostachys, R. rubritinctus* and *R. erythrops*.

The relative conservatism of the different models in predictions was not always the same for different species illustrating the value of comparing different models and examining the extent of agreement among models. For example MAXENT, was more conservative than the other models in predicting the potential distribution of *R. anglocandicans* in South Australia and in this instance failed to predict the occurrence of plants on Kangaroo Island where a few specimens have been collected (Fig. 2.3). Comparatively, MAXENT predictions for the larger southern Australian extent included areas similar to the DOMAIN model output in both location and extent (Fig. 2.2 *B* & *E*).

Advantages of multiple models

The use of multiple models allows us to examine and overcome some of the deficiencies inherent in single models particularly when working with current distribution data and generate greater confidence in the biological realism needed for application of SDM to biodiversity management. In our study, using different models rather than a combined model for each species, allows comparison of potential new invasive ranges across models that vary in their robustness and conservatism, giving land managers more complete information about the potential spread of each species and whether local areas may be at risk of invasion, particularly at the margins of known occurrences (Elith *et al.*, 2010).

Linking SDM to blackberry biology

Blackberries occupy a diverse climatic range and the choice of the predictor variables is viewed as being one of the most important elements of developing robust prediction of a species potential distribution as they represent some of the underlying niche preferences of a species (Austin *et al.*, 2006; Hirzel & Le Lay, 2008).

The climatic variables, which were used to predict the potential distribution of blackberries, also represent a subset of the biotic and abiotic niche dimensions of each species' potential distribution and the way in which these relate to the growth and survival of blackberries may help in developing a better understanding of blackberry ecology as a whole (Franklin, 2009). For example, important variables, which explain the presence of blackberries in the landscape, are the maximum temperature for June and the minimum temperature for July (Table 2.2). These variables are related to, the vernalisation period required by blackberries necessary for flowering and seed set (e.g. days below 5-10°C/year) which directly affects the ability of taxa to reproduce and expand their range (Clark *et al.*, 2007). Likewise, the potential evapotranspiration (PET) reflects the rate of evaporation and plant transpiration (Asbjornsen *et al.*, 2011).

The predictions of SDM though remain a static representation of the potential of a species to expand their populations in the landscape based upon a set of biotic and abiotic predictors and are often a first step in further studies of individual species to describe their underlying niche.

SDM in the context of management

The use of multiple models allowed us to examine and partly overcome some of the deficiencies inherent in single models and generate greater confidence in the applicability of the predictions to biodiversity management. As a management tool, SDM offers a cost-effective method to assess the potential of invasive species to invade habitat. However, to be effective SDM must be ecologically realistic and the linkages between niche theory and the associated mechanistic elements of the target organism must be part of developing models. In this study, species have shown some variation in habitat preferences across two different extents. Models of localised species such as *R. leucostachys* and *R. rubritinctus* show a potential range spread over large areas of southern Australia. The modelled bio-climatic envelope of *R. rubritinctus* is extensive, particularly for regions in south-western Western Australia and south-western Victoria suggesting that this species should be a focus for future studies of the invasive spread of blackberries in southern Australia. Contemporary management and control of introduced blackberry taxa within Australia has treated the aggregate as a whole, without considering species-by-species variation in invasive potential based on the biology and ecology of the taxa in differing habitats. Investigating and establishing the species ecological preferences and then applying the results to adaptive management may be more effective in eradication at a local level in dealing with problem species such as *R. leucostachys.*

Concluding remarks

Our approach using the multiple model framework where models have been compared and validated across spatial scales, moves away from a single model to address some of the failures identified by Austin (2007). In describing the climatic niches of the different species in this way, we attempt to give a comparative assessment for refining the management and control of these invasive species.

The protocol developed in this study allows us to combine the power of several algorithms not only to assess the way in which the predictor variables contribute to model outcomes used in SDM but also to create a suite of comparative map outputs to assess potential population expansion of individual blackberry species in Australia at different extents.

Results give a valuable insight into each species potential for population expansion at a local scale that provides land managers with valuable information for consideration in adaptive management of blackberries on a species-by-species basis.

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Appendix (App.) Chapter 2

Southern Australian extent

Supplementary data are included here for six blackberry taxa (*R. anglocandicans, R. leucostachys, R. erythrops, R. rubritinctus, R. laciniatus* and *R. ulmifolius*) for southern Australia. Each species is modelled using the five models (GLM, BIOCLIM, DOMAIN Mahalanobis and MAXENT) used above and included in the summary Fig. 2.2 and two additional geographic models, the convex hull and a geographic distance model, are included. The latter two models, (App. Figs. A2.1-A2.12), assign polygons around known locations of individual taxa in the model. These are sometimes assumed to represent a null model in geographic space (Hijmans, 2012). The geographic distance maps define a buffer around actual locations data for blackberry taxa and we used it simply to represent actual occurrence distributions more clearly in geographic space.

Each map displays the predicted ranges derived from the suite of predictor variables employed and the statistical summaries for the GLM (logistic regression) models are provided, indicating the contribution of each predictor variable to the regression model. The Akaike scores (AIC) are a measure of the fit of the model developed for each species. The area under the curve (AUC) is another measure of model fit and performance, with values near one indicating a robust model.

Supplementary Tables and Figures, Southern Australia

Table A2.1 Generalised linear model (GLM) *Rubus anglocandicans*-southern Australia

Deviance Residuals:

Min -2.2431; 1Q -0.2972; Median -0.1756; 3Q -0.1209; Max 3.2882

Coefficients:	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**
(Intercept)	9.67E+00	8.95E-01	10.801	< 2e-16
Annual aridity index	-5.30E-04	3.82E-05	-13.874	< 2e-16
PET_Dec.	-7.75E-02	1.04E-02	-7.420	1.17e-13
Precipitation_Jul.	4.28E-02	2.22E-03	19.306	< 2e-16
Solar input Aug.	3.25E-01	7.19E-02	4.528	5.95e-06
Temp. Max_Jun.	-4.95E-02	8.00E-03	-6.193	5.92e-10
Temp. Mean_Jan.	2.32E-02	9.47E-03	2.450	0.0143
Temp. Min_Jul.	3.02E-03	8.12E-03	0.372	0.7101

Null deviance: 3877.3 on 5601 degrees of freedom

Residual deviance: 2379.0 on 5594 degrees of freedom

Akaike (AIC): 2395

Number of Fisher Scoring iterations: 6

Figure A2.1 GLM - Model Evaluation

n presences : 612

n absences : 4981

Area under curve (AUC): 0.8988255

Figure A2.2 *Rubus anglocandicans*-southern Australia cont.

BIOCLIM - Model Evaluation *n* presences : 612 *n* absences : 4981 AUC : 0.93399 cor : 0.6206858 	**MAXENT- Model Evaluation** *n* presences : 612 *n* absences : 4981 AUC : 0.9563508 cor : 0.6984264 
DOMAIN - Model Evaluation *n* presences : 612 *n* absences : 4981 AUC : 0.9055486 cor : 0.4610179 	**CONVEX HULL - Model Evaluation** *n* presences : 632 *n* absences : 5000 AUC : 0.590662 cor : 0.1516367 
MAHAL - Model Evaluation *n* presences : 612 *n* absences : 4981 AUC : 0.9608076 cor : 0.3483527 	**GEOGRAPHIC DIST. - Model Evaluation** *n* presences : 632 *n* absences : 5000 AUC : 0.9735405 cor : 0.4413992 

Table A2.2 Generalised linear model (GLM) *Rubus leucostachys*-southern Australia

Deviance Residuals:

Min -1.4051; 1Q -0.1735; Median -0.0854; 3Q -0.0442; Max 3.2794

Coefficients:	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**
(Intercept)	1.28E+01	1.401E+00	9.127	< 2e-16
Annual aridity index	-5.29E-04	6.24E-05	-8.478	< 2e-16
PET_Dec.	-3.53E-02	1.53E-02	-2.308	0.02100
Precipitation_Jul.	3.31E-02	3.59E-03	9.240	< 2e-16
Solar input Aug.	-1.95E-01	1.17E-01	-1.672	0.09455
Temp. Max_Jun.	-8.31E-02	1.47E-02	-5.667	1.45e-08
Temp. Mean_Jan.	1.51E-02	1.42E-02	1.066	0.28653
Temp. Min_Jul.	3.81E-02	1.37E-02	2.774	0.00554

Null deviance: 1501.3 on 5157 degrees of freedom

Residual deviance: 1024.7 on 5150 degrees of freedom

Akaike (AIC): 1040.7

Number of Fisher Scoring iterations: 8

Figure A2.3 GLM - Model Evaluation

n presences : 174

n absences : 4981

Area under curve (AUC): 0.9182237

Pearson correlation (cor): 0.2995533

Figure A2.4 *Rubus leucostachys*-southern Australia cont.

BIOCLIM - Model Evaluation *n* presences: 174 *n* absences: 4981 AUC: 0.9332533 cor: 0.4286139 	**MAXENT - Model Evaluation** *n* presences: 174 *n* absences: 4981 AUC: 0.9615308 cor: 0.5729386 
DOMAIN - Model Evaluation *n* presences: 174 *n* absences: 4981 AUC: 0.8886712 cor: 0.2675292 	**CONVEX HULL - Model Evaluation** *n* presences: 186 *n* absences: 5000 AUC: 0.7743355 cor: 0.2048927 
MAHAL - Model Evaluation *n* presences: 174 *n* absences: 4981 AUC: 0.972417 cor: 0.2220817 	**GEOGRAPHIC DIST. - Model Evaluation** *n* presences: 186 *n* absences: 5000 AUC: 0.9817032 cor: 0.51927 

Table A2.3 Generalised linear model (GLM) *Rubus erythrops*-southern Australia

Deviance Residuals:

Min -1.8431; 1Q -0.0256; Median -0.0035; 3Q -0.0006; Max 4.2520

Coefficients:	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**
(Intercept)	23.6514724	5.36758	4.406	1.05e-05
Annual aridity index	-0.0012449	0.00023	-5.348	8.88e-08
PET_Dec.	0.0912409	0.04128	2.210	0.02710
Precipitation_Jul.	0.0781453	0.01109	7.046	1.85e-12
Solar input Aug.	-0.0775706	0.35071	-0.221	0.82495
Temp. Max_Jun.	-0.23576	0.04061	-5.805	6.42e-09
Temp. Mean_Jan.	-0.0936795	0.03489	-2.685	0.00726
Temp. Min_Jul.	0.2001174	0.04372	4.577	4.71e-06

Null deviance: 569.96 on 5037 degrees of freedom

Residual deviance: 280.74 on 5030 degrees of freedom

Akaike (AIC): 296.74

Number of Fisher Scoring iterations: 11

Figure A2.5 GLM - Model Evaluation

n presences : 51

n absences : 4974

Area under curve (AUC): 0.9693938

Pearson correlation (cor): 0.2027758

Figure A2.6 *Rubus erythrops*-southern Australia cont.

BIOCLIM - Model Evaluation *n* presences : 51 *n* absences : 4974 AUC : 0.9500934 cor : 0.6941386 	**MAXENT - Model Evaluation** *n* presences : 51 *n* absences : 4974 AUC : 0.994355 cor : 0.7620848 
DOMAIN - Model Evaluation *n* presences : 51 *n* absences : 4974 AUC : 0.9742918 cor : 0.3764758 	**CONVEX HULL - Model Evaluation** *n* presences : 52 *n* absences : 5000 AUC : 0.8679769 cor : 0.2304398 
MAHAL - Model Evaluation *n* presences : 51 *n* absences : 4974 AUC : 0.9920962 cor : 0.1010166 	**GEOGRAPHIC DIST. - Model Evaluation** *n* presences : 52 *n* absences : 5000 AUC : 0.9947115 cor : 0.5204278 

Table A2.4 Generalised linear model (GLM) *Rubus rubritinctus*-southern Australia

Deviance Residuals:

Min -1.9466; 1Q -0.0469; Median -0.0111; 3Q -0.0028; Max 3.4806

Coefficients:	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**
(Intercept)	32.0860974	4.57792	7.009	2.40e-12
Annual aridity index	-0.0020141	0.00026	-7.691	1.46e-14
PET_Dec.	0.0041647	0.0317	0.131	0.89548
Precipitation_Jul.	0.1026642	0.01092	9.397	< 2e-16
Solar input Aug.	0.2216379	0.24453	0.906	0.36472
Temp. Max_Jun.	-0.1910895	0.03202	-5.969	2.39e-09
Temp. Mean_Jan.	-0.0684198	0.02921	-2.343	0.01915
Temp. Min_Jul.	0.091298	0.02897	3.151	0.00163

Null deviance: 780.69 on 5061 degrees of freedom

Residual deviance: 405.01 on 5054 degrees of freedom

Akaike (AIC): 421.01

Number of Fisher Scoring iterations: 9

Figure A2.7 GLM - Model Evaluation

n presences : 76

n absences : 4974

Area under curve (AUC): 0.977557

Pearson correlation (cor): 0.2328327

Figure A2.8 *Rubus rubritinctus*-southern Australia cont.

BIOCLIM - Model Evaluation *n* presences : 76 *n* absences : 4974 AUC : 0.9836386 cor : 0.802987 	**MAXENT - Model Evaluation** *n* presences : 76 *n* absences : 4974 AUC : 0.9948257 cor : 0.8898089 
DOMAIN - Model Evaluation *n* presences : 76 *n* absences : 4974 AUC : 0.9944091 cor : 0.48826 	**CONVEX HULL - Model Evaluation** *n* presences : 76 *n* absences : 5000 AUC : 0.9583211 cor : 0.4014767 
MAHAL - Model Evaluation *n* presences : 76 *n* absences : 4974 AUC : 0.9988837 cor : 0.0704865 	**GEOGRAPHIC DIST.- Model Evaluation** *n* presences : 76 *n* absences : 5000 AUC : 0.9998553 cor : 0.5688701 

Table A2.5 Generalised linear model (GLM) *Rubus laciniatus*-southern Australia

Deviance Residuals:

Min -1.2671; 1Q -0.0896; Median -0.0408; 3Q -0.0226; Max 4.2567

Coefficients:	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**
(Intercept)	3.69E-02	2.70E+00	0.014	0.989085
Annual aridity index	-2.43E-04	8.76E-05	-2.776	0.005502
PET_Dec.	6.22E-02	3.03E-02	2.053	0.040101
Precipitation_Jul.	3.15E-02	5.34E-03	5.890	3.86e-09
Solar input Aug.	5.53E-01	2.43E-01	2.278	0.022726
Temp. Max_Jun.	-1.25E-01	3.08E-02	-4.064	4.83e-05
Temp. Mean_Jan.	-5.09E-02	2.84E-02	-1.792	0.073155
Temp. Min_Jul.	1.21E-01	3.15E-02	3.828	0.000129

Null deviance: 476.02 on 5027 degrees of freedom

Residual deviance: 330.78 on 5020 degrees of freedom

Akaike (AIC): 346.78

Number of Fisher Scoring iterations: 9

Figure A2.9 GLM - Model Evaluation

n presences : 41

n absences : 4974

Area under curve (AUC): 0.9180813

Pearson correlation (cor): 0.1923836

Figure A2.10 *Rubus laciniatus*-southern Australia cont.

BIOCLIM - Model Evaluation *n* presences : 41 *n* absences : 4974 AUC : 0.9289451 cor : 0.4493606 	**MAXENT - Model Evaluation** *n* presences : 41 *n* absences : 4974 AUC : 0.9780713 cor : 0.565196 
DOMAIN - Model Evaluation *n* presences : 41 *n* absences : 4974 AUC : 0.9556253 cor : 0.2081239 	**CONVEX HULL - Model Evaluation** *n* presences : 41 *n* absences : 5000 AUC : 0.8181195 cor : 0.128512 
MAHAL - Model Evaluation *n* presences : 41 *n* absences : 4974 AUC : 0.9569665 cor : 0.1377319 	**GEOGRAPHIC DIST. - Model Evaluation** *n* presences : 41 *n* absences : 5000 AUC : 0.9852927 cor : 0.484138 

Table A2.6 Generalised linear model (GLM) *Rubus ulmifolius var. ulmifolius* -southern Australia

Deviance Residuals:

Min -2.0003; 1Q -0.1230; Median -0.0603; 3Q -0.0223; Max 4.6513

Coefficients:	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**
(Intercept)	15.5421026	2.63672	5.894	3.76e-09
Annual aridity index	-0.0010336	0.00015	-6.761	1.37e-11
PET_Dec.	-0.0223873	0.02272	-0.985	0.32447
Precipitation_Jul.	0.0572779	0.00626	9.145	< 2e-16
Solar input Aug.	0.5019367	0.17302	2.901	0.00372
Temp. Max_Jun.	-0.1917353	0.02239	-8.563	< 2e-16
Temp. Mean_Jan.	0.0144844	0.01897	0.763	0.44525
Temp. Min_Jul.	0.105441	0.02126	4.959	7.09e-07

Null deviance: 1137.37 on 5106 degrees of freedom

Residual deviance: 680.82 on 5099 degrees of freedom

Akaike (AIC): 696.82

Number of Fisher Scoring iterations: 9

Figure A2.11 GLM - Model Evaluation

n presences : 122

n absences : 4981

Area under curve (AUC): 0.9125398

Pearson correlation (cor): 0.2471581

Figure A2.12 *Rubus ulmifolius var. ulmifolius* -southern Australia

BIOCLIM - Model Evaluation *n* presences : 122 *n* absences : 4981 AUC : 0.9150551 cor : 0.4884815 	**MAXENT- Model Evaluation** *n* presences : 122 *n* absences : 4981 AUC : 0.9601296 cor : 0.5114731 
DOMAIN - Model Evaluation *n* presences : 122 *n* absences : 4981 AUC : 0.9130154 cor : 0.2479323 	**CONVEX HULL - Model Evaluation** *n* presences : 122 *n* absences : 5000 AUC : 0.6109164 cor : 0.07391938 
MAHAL - Model Evaluation *n* presences : 122 *n* absences : 4981 AUC : 0.9716867 cor : 0.139851 	**GEOGRAPHIC DIST. - Model Evaluation** *n* presences : 122 *n* absences : 5000 AUC : 0.9730033 cor : 0.5371798 

Supplementary Tables and Figures, South Australia

South Australian extent

Supplementary data for six blackberry taxa (*R. anglocandicans, R. leucostachys, R. erythrops*, *R. rubritinctus, R. laciniatus* and *R. ulmifolius* var. *ulmifolius*) taxa for South Australia are presented in the same way as the data for southern Australia.

Models for *R. erythrops* are likely to be less reliable, due to the small data set (App. Table A2. 9; Fig. A2.17; Fig. A2.18).

Table A2.7 Generalised linear model (GLM) *Rubus anglocandicans*-South Australia

Deviance Residuals:

Min -2.4505; 1Q -0.0905; Median -0.0478; 3Q -0.0211; Max 3.2221

Coefficients:	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**
(Intercept)	17.2914527	10.022426	1.725	0.0845
Annual aridity index	-0.0001116	0.0003512	-0.318	0.7507
PET_Dec.	-0.2878525	0.0576163	-4.996	5.85e-07
Precipitation_Dec.	0.3737419	0.0562139	6.649	2.96e-11
Solar input Oct.	-1.7623264	0.3411099	-5.166	2.39e-07
Temp. Max_Jun.	0.2420569	0.0547188	4.424	9.70e-06
Temp. Mean_Jan.	0.1646293	0.0412948	3.987	6.70e-05
Temp. Min_Jul.	-0.2893972	0.0563941	-5.132	2.87e-07

Null deviance: 1275.07 on 5088 degrees of freedom

Residual deviance: 485.93 on 5081 degrees of freedom

Akaike (AIC): 501.93

Number of Fisher Scoring iterations: 9

Figure A2.13 GLM - Model Evaluation

n presences : 139

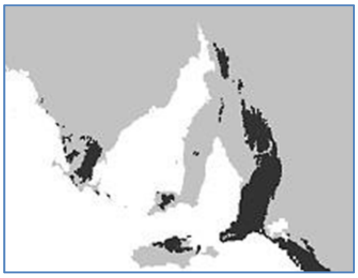
n absences : 4950

Area under curve (AUC): 0.9813873

Pearson correlation (cor): 0.459439

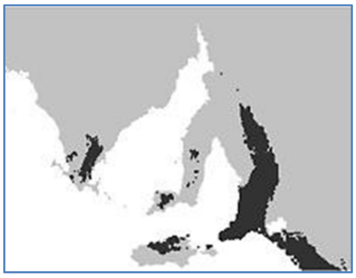
Figure A2.14 *Rubus anglocandicans*-South Australia cont.

BIOCLIM - Model Evaluation
n presences : 139
n absences : 4950
AUC : 0.9691585
cor : 0.5707649

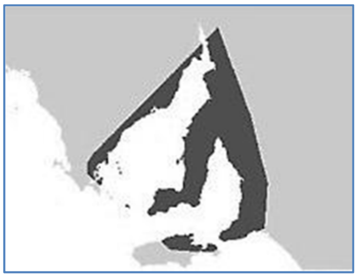


MAXENT- Model Evaluation
n presences : 139
n absences : 4950
AUC : 0.9878199
cor : 0.7386644

DOMAIN - Model Evaluation
n presences : 139
n absences : 4950
AUC : 0.3583607
cor : 0.3583607



CONVEX HULL - Model Evaluation
n presences : 140
n absences : 5000
AUC : 0.9035857
cor : 0.3326649



MAHAL - Model Evaluation
n presences : 139
n absences : 4950
AUC : 0.4867705
cor : 0.2048311

GEOGRAPHIC DIST. - Model Evaluation
n presences : 140
n absences : 5000
AUC : 0.9882486
cor : 0.3824539

Table A2.8 Generalised linear model (GLM) *Rubus leucostachys*-South Australia

Deviance Residuals:

Min -1.9950; 1Q -0.0609; Median-0.0308; 3Q -0.0181; Max 3.4635

Coefficients:	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**
(Intercept)	21.4240892	14.986255	1.430	0.152837
Annual aridity index	-0.0002131	0.0005466	-0.390	0.696681
PET_Dec.	-0.0981864	0.0860261	-1.141	0.253722
Precipitation_Dec.	0.2832897	0.0767652	3.690	0.000224
Solar input Oct.	-1.8730275	0.5285936	-3.543	0.000395
Temp. Max_Jun.	-0.0397331	0.092044	-0.432	0.665977
Temp. Mean_Jan.	0.1511026	0.0657871	2.297	0.021628
Temp. Min_Jul.	-0.06455	0.0872413	-0.740	0.459360

Null deviance: 522.94 on 5000 degrees of freedom

Residual deviance: 255.07 on 4993 degrees of freedom

Akaike (AIC): 271.07

Number of Fisher Scoring iterations: 10

Figure A2.15 GLM - Model Evaluation

n presences : 46

n absences : 4945

Area under curve (AUC): 0.9688882

Pearson correlation (cor): 0.2626134

Figure A2.16 *Rubus leucostachys*-South Australia cont.

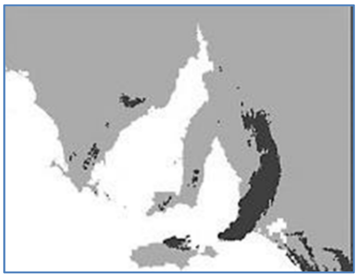
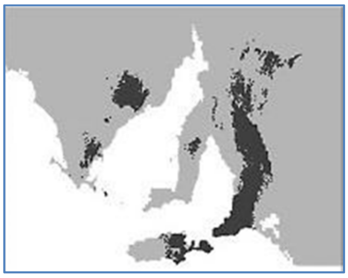
BIOCLIM - Model Evaluation *n* presences : 46 *n* absences : 4945 AUC : 0.9169495 cor : 0.4623729 	**MAXENT - Model Evaluation** *n* presences : 46 *n* absences : 4945 AUC : 0.9774783 cor : 0.44899 
DOMAIN - Model Evaluation *n* presences : 46 *n* absences : 4945 AUC : 0.9396162 cor : 0.2300315 	**CONVEX HULL - Model Evaluation** *n* presences : 46 *n* absences : 5000 AUC : 0.8501739 cor : 0.2124102 
MAHAL - Model Evaluation *n* presences : 46 *n* absences : 4945 AUC : 0.9734339 cor : 0.1259634 	**GEOGRAPHIC DIST. - Model Evaluation** *n* presences : 46 *n* absences : 5000 AUC : 0.9778522 cor : 0.456319 

Table A2.9 Generalised linear model (GLM) *Rubus erythrops*-South Australia

Deviance Residuals:

Min -1.5169; 1Q -0.0055; Median -0.0018; 3Q -0.0007; Max 3.2155

Coefficients:	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**
(Intercept)	13.2370618	49.699531	0.266	0.790
Annual aridity index	0.0009293	0.0014767	0.629	0.529
PET_Dec.	0.2285462	0.3194027	0.716	0.474
Precipitation_Dec.	0.1190444	0.2051739	0.580	0.562
Solar input Oct.	-2.2639545	2.0148694	1.124	-0.261
Temp. Max_Jun.	-0.3274046	0.3063259	-1.069	0.285
Temp. Mean_Jan.	0.0627063	0.2482993	0.253	0.801
Temp. Min_Jul.	0.2237086	0.3223457	0.694	0.488

Null deviance: 249.502 on 4974 degrees of freedom

Residual deviance: 62.386 on 4967 degrees of freedom

Akaike (AIC): 78.386

Number of Fisher Scoring iterations: 13

Figure A2.17 GLM - Model Evaluation

n presences : 18

n absences : 4949

Area under curve (AUC): 0.9972385

Pearson correlation (cor): 0.2350389

Figure A2.17 *Rubus erythrops*-South Australia cont.

BIOCLIM - Model Evaluation *n* presences : 18 *n* absences : 4949 AUC : 0.8586527 cor : 0.4903577 	**MAXENT - Model Evaluation** *n* presences : 18 *n* absences : 4949 AUC : 0.9963405 cor : 0.6852638 
DOMAIN - Model Evaluation *n* presences : 18 *n* absences : 4945 AUC : 0.995935 cor : 0.9001889 	**CONVEX HULL - Model Evaluation** *n* presences : 19 *n* absences : 5000 AUC : 0.8404053 cor : 0.5424013 
MAHAL - Model Evaluation *n* presences : 18 *n* absences : 4945 AUC : 0.9920958 cor : 0.3989345 	**GEOGRAPHIC DIST. – Model Evaluation** *n* presences : 19 *n* absences : 5000 AUC : 0.9871684 cor : 0.4175493 

Table A2.10 Generalised linear model (GLM) *Rubus rubritinctus*-South Australia

Deviance Residuals:

Min -2.3126; 1Q -0.0280; Median -0.0140; 3Q -0.0067; Max 3.8050

Coefficients:	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**
(Intercept)	21.7568048	21.3106946	1.021	0.307286
Annual aridity index	0.0005671	0.0008193	0.692	0.488813
PET_Dec.	-0.0312313	0.1404558	-0.222	0.824036
Precipitation_Dec.	0.2080922	0.1425875	1.459	0.144455
Solar input Oct.	-2.6295151	0.7066227	-3.721	0.000198
Temp. Max_Jun.	0.0434213	0.1555104	0.279	0.780077
Temp. Mean_Jan.	0.1203441	0.0850559	1.415	0.157103
Temp. Min_Jul.	-0.1783851	0.1455488	-1.226	0.220348

Null deviance: 446.44 on 4991 degrees of freedom

Residual deviance: 156.27 on 4984 degrees of freedom

Akaike (AIC): 172.27

Number of Fisher Scoring iterations: 11

Figure A2.18 GLM - Model Evaluation

n presences : 40

n absences : 4950

Area under curve (AUC): 0.991899

Pearson correlation (cor): 0.3092395

Figure A2.18 *Rubus rubritinctus*-South Australia cont.

BIOCLIM - Model Evaluation

n presences : 40
n absences : 4950
AUC : 0.9802525
cor : 0.5709194

MAXENT - Model Evaluation

n presences : 40
n absences : 4950
AUC : 0.9953131
cor : 0.6311698

DOMAIN - Model Evaluation

n presences : 40
n absences : 4950
AUC : 0.9866364
cor : 0.3457587

CONVEX HULL - Model Evaluation

n presences : 40
n absences : 5000
AUC : 0.9121
cor : 0.3145619

MAHAL - Model Evaluation

n presences : 40
n absences : 4950
AUC : 0.9932475
cor : 0.07456283

GEOGRAPHIC DIST. - Model Evaluation

n presences : 40
n absences : 5000
AUC : 0.996695
cor : 0.3950324

Table A2.11 Generalised linear model (GLM) *Rubus laciniatus*-South Australia

Deviance Residuals:

Min -2.30104; 1Q -0.00545; Median -0.00158; 3Q -0.00032; Max 2.32037

Coefficients:	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**
(Intercept)	-7.138414	42.121578	-0.169	0.86543
Annual aridity index	0.001205	0.001739	0.693	0.48844
PET_Dec.	0.531381	0.298067	1.783	0.07463
Precipitation_Dec.	0.065576	0.279888	0.234	0.81476
Solar input Oct.	-1.779698	1.602237	-1.111	0.26667
Temp. Max_Jun.	-0.810692	0.306353	-2.646	0.00814
Temp. Mean_Jan.	0.107499	0.205814	0.522	0.60145
Temp. Min_Jul.	0.594604	0.283620	2.096	0.03604

Null deviance: 335.623 on 4982 degrees of freedom

Residual deviance: 59.219 on 4975 degrees of freedom

Akaike (AIC): 75.219

Number of Fisher Scoring iterations: 13

Figure A2.19 GLM - Model Evaluation

n presences : 26

n absences : 4965

Area under curve (AUC): 0.9958479

Pearson correlation (cor): 0.2371379

Figure A2.19 *Rubus laciniatus*-South Australia cont.

BIOCLIM - Model Evaluation *n* presences : 26 *n* absences : 4965 AUC : 0.9591409 cor : 0.6609097 	**MAXENT- Model Evaluation** *n* presences : 26 *n* absences : 4965 AUC : 0.9913278 cor : 0.7413501 
DOMAIN - Model Evaluation *n* presences : 26 *n* absences : 4965 AUC : 0.9778178 cor : 0.5874056 	**CONVEX HULL - Model Evaluation** *n* presences : 26 *n* absences : 5000 AUC : 0.8817154 cor : 0.5573904 
MAHAL - Model Evaluation *n* presences : 26 *n* absences : 4965 AUC : 0.9959912 cor : 0.09781844 	**GEOGRAPHIC DIST. - Model Evaluation** *n* presences : 26 *n* absences : 5000 AUC : 0.9965615 cor : 0.5547345 

Table A2.12 Generalised linear model (GLM) *Rubus ulmifolius* var. *ulmifolius*-South Australia

Deviance Residuals:

Min 1.3333; 1Q -0.0276; Median -0.0100; 3Q -0.0031; Max 3.5567

Coefficients:	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**
(Intercept)	66.6895211	21.5109268	3.100	0.00193
Annual aridity index	-0.0018541	0.0009629	-1.925	0.05418
PET_Dec.	-0.0291618	0.1292882	-0.226	0.82155
Precipitation_Dec.	0.3160634	0.1475072	2.143	0.03214
Solar input Oct.	-2.4606025	0.6161272	-3.994	6.51e-05
Temp. Max_Jun.	-0.5842961	0.1408249	-4.149	3.34e-05
Temp. Mean_Jan.	0.2834641	0.1125610	2.518	0.01179
Temp. Min_Jul.	0.1752709	0.1195977	1.466	0.14278

Null deviance: 303.91 on 4973 degrees of freedom

Residual deviance: 156.07 on 4966 degrees of freedom

Akaike (AIC): 172.07

Number of Fisher Scoring iterations: 11

Figure A2.20 GLM - Model Evaluation

n presences : 24

n absences : 4958

Area under curve (AUC): 0.9733764

Pearson correlation (cor): 0.1665697

Figure A2.20 *Rubus ulmifolius var. ulmifolius* -South Australia cont.

BIOCLIM - Model Evaluation *n* presences : 24 *n* absences : 4958 AUC : 0.8727688 cor : 0.2165754 	**MAXENT - Model Evaluation** *n* presences : 24 *n* absences : 4958 AUC : 0.9747588 cor : 0.4217929 
DOMAIN - Model Evaluation *n* presences : 24 *n* absences : 4958 AUC : 0.9619344 cor : 0.1876771 	**CONVEX HULL - Model Evaluation** *n* presences : 24 *n* absences : 5000 AUC : 0.8574 cor : 0.2558001 
MAHAL - Model Evaluation *n* presences : 24 *n* absences : 4958 AUC : 0.9518203 cor : 0.08526248 	**GEOGRAPHIC DIST. - Model Evaluation** *n* presences : 24 *n* absences : 5000 AUC : 0.9525667 cor : 0.4095073 

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Chapter 3

Testing niche differentiation among species of the *Rubus fruticosus* L. aggregate in South Australia

[1]John Marshall, [2] Molly A. Whalen, [3] and Duncan A. Mackay

[1, 2, 3] Flinders University, School of Biological Sciences, GPO Box 2100, Adelaide, S.A. 5001, Australia.

Abstract

Aim We aim to measure niche similarity and niche differences along a series of bio-climatic axes between closely related species of European blackberries, which occupy different but often overlapping geographic distributions.

Location Mt. Lofty Ranges, South Australia

Methods We used the outputs of species distribution models for six invasive blackberries found within the Mt. Lofty Ranges and estimated their pair-wise niche similarities. We used the ENMTools software to calculate measures of niche identity, niche overlap and niche background. Significance tests for paired comparisons (two tailed tests) between the six blackberry species were based upon the Hellinger's I_{mod}, and Schoener's D statistics.

Results The niche identity tests and similarity tests show that the majority of species appear differentiated with respect to the collections of climatic predictor variables used to build the niche models. Environmental niche models calculated for *R. anglocandicans* show the largest significant differences when compared with other species' niches based upon the underlying bio-climatic predictors.

Main Conclusions The niche overlap analyses indicate that the modelled niches of the blackberry species studies are significantly differentiated with respect to the climatic predictor variables used to build the niche models. Niche overlaps were highest between *R. anglocandicans* and *R. leucostachys* and least between *R. anglocandicans* and the other species (except for *R. phaeocarpus,* where a small dataset makes the result less reliable).

Keywords: **niche differentiation, niche conservatism, niche overlap, niche identity, MAXENT, ENMTools, adaptive shift, blackberries, *Rubus fruticosus* agg.**

Introduction

In ecological studies, species distribution modelling (SDM) is an expanding field used to enhance understanding of the underlying processes that influence species distributions in geographic and environmental space. In ecological theory, the fundamental niche of a species (as estimated by SDM) is characterised by the abiotic conditions in which a species is able to persist, whereas the original or realized niche reflects the environment in which a species actually exists with predator and competitor species (Hutchinson, 1957). Using SDM to describe the fundamental niche is a useful tool for biodiversity management and offers predictive assessment of a species' potential range in both native and introduced ranges. Maps generated are based upon actual occurrences extrapolated using data from predictor (typically climatic) variables. SDM outputs may then be used by land managers for biodiversity assessment, habitat management, community and ecosystem modelling and invasive species risk assessment (Franklin, 2009).

Invasive species by definition are adaptable and superior competitors in new ranges and some evidence supports the premise that introduced weeds undergo a shift in their climatic tolerances which allows them to expand their populations but the rate, degree and mechanisms of adaptation of species within Australia and elsewhere remains little known and under-represented in the literature (Beaumont *et al.*, 2009). Measuring the adaptability of invasive taxa in new ranges is of great interest to land managers involved with biodiversity conservation but requires considerable knowledge of the biotic and abiotic preferences of invasive taxa from their original niches. Home range data for most weed taxa though are often unobtainable or incomplete, which limits the interpretation of SDMs when examining whether invasive species are extending their climatic tolerances and adaptation in new ranges.

Some workers have suggested that invasive weeds may display 'niche conservatism', defined by Wiens and Graham (2005) as the tendency of a species to keep characteristics of their original or realised niche over time and have concluded that conservatism in climatic tolerances limits the expansion of geographic ranges of species and clades, such that species will only be able to invade regions that have a climate similar to that of their native range. Broennimann *et al.* (2007) showed that the invasive spotted knapweed (*Centaurea maculosa* L.) in western North America and Europe conserved their climatic niche in the invaded ranges by using SDM calibrated to the climatic characteristics of the home range of the species.

Despite improvements in our ability to model species distributions (Guisan & Thuiller, 2005), most modelled ranges still represent a static model. Little attention has been applied to developing techniques to quantify other characteristics of an invasive species' niche that could enhance our understanding of invasive species (Warren *et al.*, 2008).

One solution suggested by Townsend Peterson (2003) where closely related species exist in mixed and overlapping populations is to use environmental niche modelling to quantify the niche differentiation among species to assess the differences among species niches which may indicate if species are adapting within their new ranges (Townsend Peterson & Vieglais, 2001; Townsend Peterson *et al.*, 2003).

Aims

We aim to measure the extent of niche differentiation between closely related species of European blackberries that are invasive in Australia. We also ask whether the patterns of niche differentiation likely reflect adaptations to different Australian conditions.

Problematic introduced blackberry weeds

European blackberries of the *Rubus fruticosus* L. aggregate are one of Australia's worst weeds and adaptable to a broad range of habitats and climatic conditions (Agriculture & Resource Management Council of Australia & New Zealand & Australian & New Zealand Environment & Conservation Council and Forestry Ministers, 2000). Commercial evidence shows species of *R. fruticosus* were introduced into South Australia between 1857 and 1900 for horticulture (Giles & Pascoe, 1868; Newman, 1893).

Ten species occur in the Mt. Lofty Ranges (Fig. 3.1) where populations display differing spatial extents but sometimes in mixed populations, suggesting either they have discrete but overlapping habitat preferences or that they have not reached the limit of their invasive ranges due to other factors such as time since introduction. Knowledge of the ecological and biological preferences of most taxa within the aggregate in the introduced ranges within Australia and South Australia is incomplete, but a recent revision of the taxonomy of Australian taxa has helped in providing the basis for more extended study of these taxa (Barker & Barker, 2005; Evans *et al.*, 2007).

One of the most studied species in the *R. fruticosus* group, *Rubus anglocandicans* A. Newton, is the most frequently encountered in the Australian landscape but few studies have characterised its invasiveness (Evans & Weber, 2003). In its native range in Great Britain, *R. anglocandicans* is a regional endemic species, essentially not widely distributed and found mostly in the East Midlands (Edees & Newton, 1988). The East Midlands is located on a high plateau with a climate profile characterised by a mean annual temperature range of minus 8°C to plus 10°C, sharp winter frosts and very hot summer days, particularly in the south and east of the region. Temperature extremes of both winter and summer are a key characteristic of the Midlands climate, with rainfall averages of 800mm per year (Met Office, 2015). In Australia, *R. anglocandicans* occupies large areas above the 760mm isohyet over a diverse temperature range characterized by hot dry summers and invades a variety of native habitats and agricultural areas (Amor, 1973; Amor & Miles, 1974). A brief comparison of the climate regimes between the native and introduced ranges would suggest *R. anglocandicans* should not be such a successful invader in the Australian landscape.

Currently, little specific ecological and biological information on the individual niche preferences within Australia of blackberry species exists, and much of what is available is based upon either home range observations or the limited studies by Amor (1973) of *R. procerus* in Australia.

Methods

Background to statistical analysis of blackberry populations

We use methods developed by Warren (2009) to measure the niche overlap and niche similarity among species of the *R. fruticosus* L., using a suite of validated bio-geographical predictor variables developed for SDM using MAXENT, available URL http://www.cs.princeton.edu/~schapire/maxent/ (Phillips *et al.*, 2004; Phillips & Schapire, 2006; Phillips & Dudik, 2008; Elith *et al.*, 2011). Calculated niche overlaps reflect the actual similarity between predictions of habitat suitability generated in MAXENT between pairs of species within the study area (Fig. 3.2). ENMTools (available URL http://enmtools.blogspot.com.au/) calculates two quantitative tests of the non-randomness or statistical significance of the observed pair-wise niche overlaps - a niche identity test and a niche background test. The former tests whether the environmental niche models (ENMs) derived from two or more blackberry populations are more different than would be expected if they were drawn from the same underlying distributions of environmental variables. It does this by randomizing the species identities of the empirical locality data. The null hypothesis is one of niche identity.

The niche background test determines whether the ENMs of two selected species are more or less similar than expected by chance given the actual geographical regions of interest in which each occurs (Fig. 3.2). It does this by randomly positioning individuals of one species within the range of another and then calculating the niche overlap.

Several species of the *R. fruticosus* agg. occur in the Mt. Lofty Ranges, South Australia, sometimes in mixed populations (Fig. 3.1, 3.2). We predict that they do not occupy identical niches in the landscape.

While we have no specific data on the bio-climatic preferences of *Rubus* taxa from their native ranges, constructing data of the individual species preferences in their invaded ranges in South Australia allows an evaluation of the extent of differentiation in the niches where they currently occur. We extracted bio-climatic data using actual occurrence points from the invaded ranges in South Australia from the predictor variables used for species distribution modelling in Chapter 2 and used the tests described above to statistically test the significance of the observed overlaps in modelled niches. These tests therefore allow us to evaluate the extent to which species in the *R. fruticosus* aggregate vary in the types of bio-climatic conditions that they currently occupy in the Mount Lofty Ranges or into which they may spread in the future.

Data sets

Species occurrence data

We obtained actual point occurrence data of blackberry species from the State Herbarium of South Australia (AD) and field collections of new material within South Australia (Mt. Lofty Ranges) (Fig. 3.1).

We used presence-only data to construct species distribution models (SDMs) using the MAXENT maximum entropy algorithm as part of distribution mapping of blackberry species within South Australia (see Chapter 2). This process provided the estimated environmental niches of each model blackberry taxa required for niche similarity testing in ENMTools (Warren *et al.*, 2010).

While native range data may be available for some plant species, there are considerable gaps in the knowledge of blackberry species in Europe for the more than 2000+ species identified. Considerable searches of plant databases within both Great Britain and Europe yielded little information, particularly for the invasive species within Australia. A complete knowledge of spatial information for most blackberry species in their native ranges is lacking and limited in its ability to be used in SDM and for generating robust and useful models.

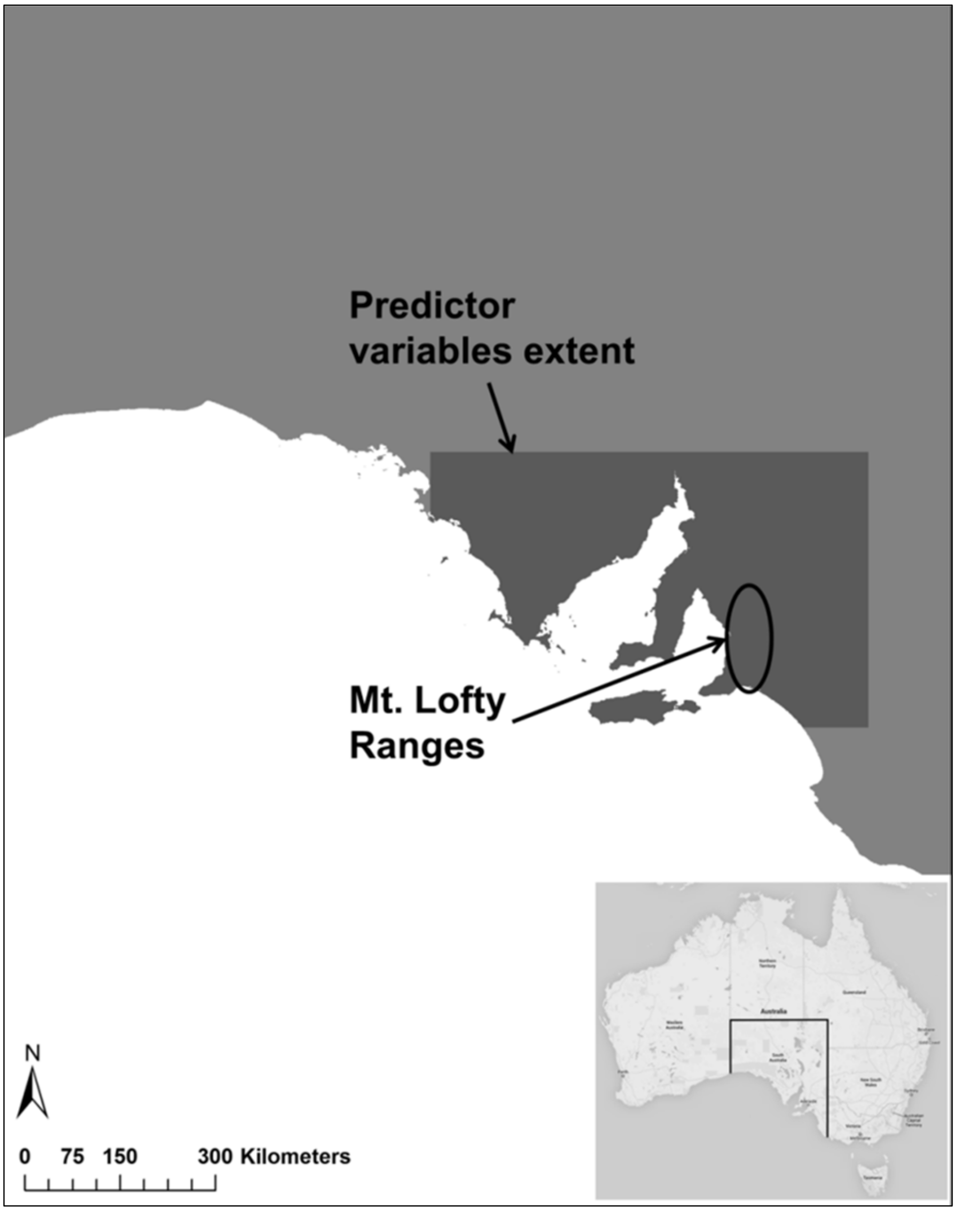

Figure 3.1 Map of South Australia showing location of the Mt. Lofty Ranges (circled) and the predictor variables extent (insert shows the state of South Australia within Australia) map elements sourced (ESRI®, 2010; Patterson & Kelso, 2013)

Predictor variables

We sourced climatic predictors from WorldClim.org, available URL (http://www.worldclim.org/) (Hijmans *et al.*, 2005). The predictor variables for aridity where higher numbers represent more humid and wetter conditions and potential evapotranspiration (PET) were sourced from CGIAR, available URL http://www.cgiar-csi.org/ (Zomer *et al.*, 2007; Zomer *et al.*, 2008). We also used a gridded solar exposure layer obtained from the Australian Bureau of Meteorology (Bureau of Meteorology, 2007). Marine masking layers to exclude the oceans in species distribution modelling and ENM were sourced from The Australian Government Department of Sustainability, Environment, Water, Population and Communities (Department of Sustainability Environment Water Population and Communities, 2011).

MAXENT was used in the species modelling study to select the variables, which contribute most (as measured by the Area Under Curve (AUC) criterion) to the output modelling of the most common species *R. anglocandicans* in Chapter 2. Seven predictor variables were identified for testing niche similarity in ENMTools. We re-sampled the predictor layers to the extent of the study area (Fig. 3.1) and interpolated these layers using GIS platforms (ESRI ArcMap v 10.0; DIVA-GIS v7.5.0), to a standard 30 second gridded resolution (Harris *et al.*, 2014).

Occurrence of *Rubus* taxa represented by polygons

The background similarity test requires data sets of the range of individual *Rubus* species, for testing pair-wise overlaps among blackberry species. We generated ranges of each species using polygons of the geographic extent for each *Rubus* taxa within the Mt. Lofty Ranges using the "aggregate points" tool in Arcmap$^{®}$ version 10, around actual occurrences of blackberry species of three or more points within an aggregation distance of 5km (Fig. 3.2). This process excluded outliers where no other occurrence point for a species occurred within a five-kilometre radius.

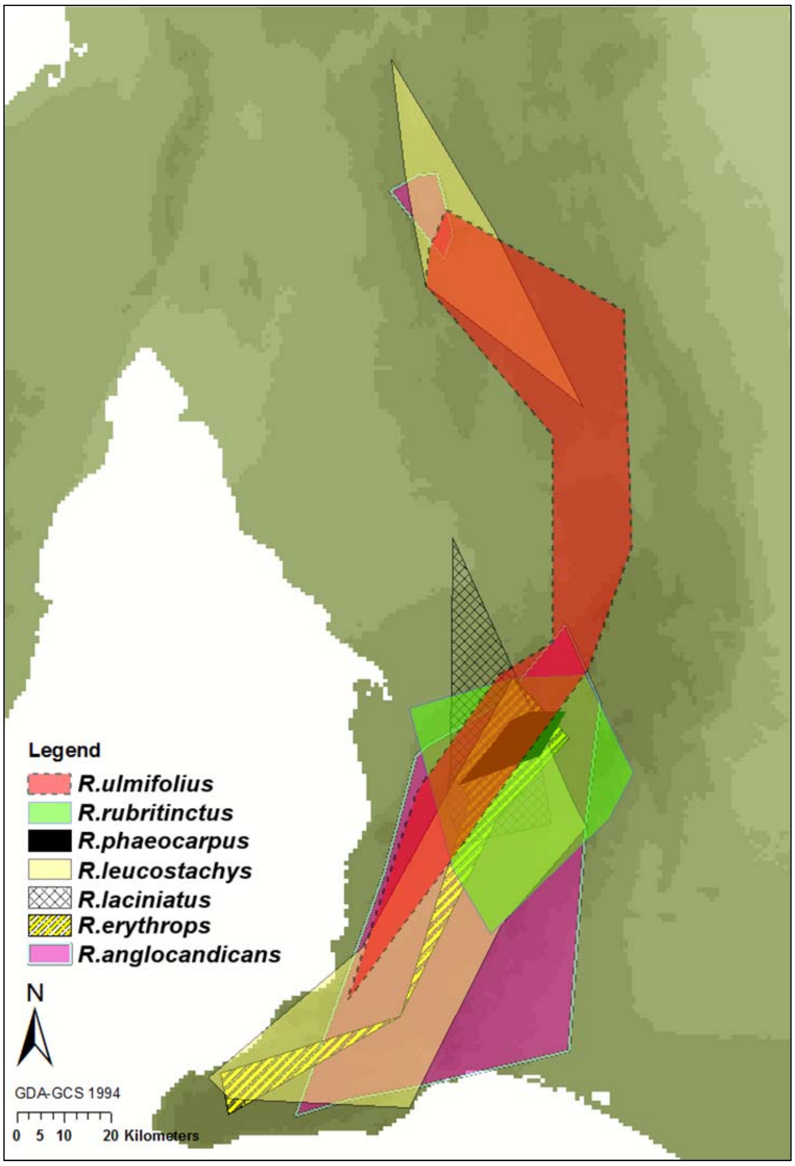

Figure 3.2 Species polygon map of *Rubus* spp. within the Mt. Lofty Ranges, South Australia used for the background test

Tests for niche similarity in ENMTools

Actual niche overlap between paired species

The niche overlap metric calculated by ENMTools (Warren *et al*. 2010) measures the similarity between predictions of suitable habitat between pairs of *Rubus* taxa from species distribution modelling derived from output files generated by MAXENT. We set the default values at 100 replicates (as recommended by Warren *et al*. (2008) and constructed the distribution models using partitioned *Rubus* occurrence data (80:20 testing and training ratio).

We performed two-tailed pair-wise niche overlaps of each species pair of blackberry taxa (Warren *et al.*, 2008). Each niche overlap test generated two different statistics, Schoener's *D* (Schoener, 1968) and the modified Hellinger's I_{mod} statistic (Warren *et al.*, 2008). The scale for all metrics ranges from zero (no niche overlap) to one (niches are identical). Schoener's *D* metric includes some biological preferences of the tested species in final metric (Schoener, 1968), whereas Hellinger's I_{mod} is a distance based measurement between random frequency distributions of paired species (modified by Warren *et al.*, 2008).

The niche identity test

The niche identity test uses MAXENT within ENMTools to build environmental niche models for pair-wise comparisons of species of *Rubus* taxa based upon the underlying predictor variables (Warren *et al.*, 2010). We compared the niche identities of paired *Rubus* taxa using five hundred pseudo-replicates to compare with the observed overlaps (Warren *et al.*, 2010).

The background test

The background test is used to determine whether the modelled ENMs of two species are more or less similar than expected by chance, given the regions in which they both occur (Warren *et al.*, 2010). The background test generates a null distribution of differences between the ENM modelled for one species in its range and that derived from occurrence points of the second species placed at random within its range (Warren *et al.*, 2010). We performed pair-wise background similarity tests between the background regions of each blackberry taxa (Fig 3.2) using 100 iterations to generate the null distribution of differences (Warren *et al.*, 2010).

Characterising the habitat preferences of *Rubus* taxa

To assist in interpreting the modelled environmental niches and their overlaps generated using species distribution models (Chapter 2) and ENMTools (this chapter), the values of the bio-climatic predictor values at the actual localities of all *Rubus* taxa were extracted. We also extracted the values of these predictor variables at 500 random locations within the geographic extent of the predictors (Fig. 3.1) for comparison.

Results

Tests for niche similarity

Results of the niche identity test

The niche identity test in ENMTools builds ENMs from actual occurrence point data and then compares the actual niche overlap scores of paired blackberry species against a random dataset drawn from resampling the populations of each species and randomising the species identities. The niche identity test quantifies the differences between the species' niches using the Hellinger's I_{mod} and Schoener's D statistics.

The results for most blackberry species pairs showed a significant difference between the randomized pair-wise overlaps and the actual observed values for both the I_{mod} and D statistics. Most pair-wise overlaps between blackberry species are significantly less than expected (Table 3.1), indicating a significant niche differentiation.

We chose the pair-wise comparisons of niche identity for *R. leucostachys* against six species for illustrative purposes, as it occupies a large range within South Australia and has a large dataset (Figs. 3.3 & 3.4).

The Hellinger's I_{mod} and Schoener's D statistics for *R. leucostachys* paired with other blackberry species demonstrated the actual niche overlap scores (arrows, Figs. 3.3 & 3.4) were significantly less than expected in five out of six paired comparisons with other blackberry taxa (Table 3.1). The pairing of *R. leucostachys vs. R. phaeocarpus* though was non-significant but should be treated with caution due to a small sample size (n=16) for the latter species in the analyses. The values of the I_{mod} and D statistics for *R. leucostachys vs. R. ulmifolius, R. erythrops, R. anglocandicans, R. laciniatus* and *R. rubritinctus* showed that the actual overlaps between these species pairs are significantly less than expected when compared to the null distribution (Figs. 3.3 & 3.4, Table 3.1).

The frequency histograms of pair-wise comparisons among other species display a similar pattern where most actual pair-wise comparisons are significantly less than expected under the random distribution (App. Figs. A3.1-A3.6).

Table 3.1 Summary table of two-tailed overlap similarity scores between *Rubus* species pairs for the Niche identity test. Asterisks indicate significance of randomisation test of the null hypothesis of random overlap

Species pairs	**Hellinger's I_{mod} Distances**	**Schoener's D Distances**
1_vs_2	0.820 ***L	0.535 ***L
1_vs_3	0.970 ns	0.844 ns
1_vs_4	**0.850 ***L**	**0.550 ***L**
1_vs_5	0.907 ns	0.660 ns
1_vs_6	0.810 *L	0.486 ***L
1_vs_7	0.995 ***G	0.665 ns
2_vs_3	0.820 ***L	0.535 ***L
2_vs_4	**0.919 *L**	**0.740 ***L**
2_vs_5	0.843 ***L	0.570 ***L
2_vs_6	0.869 ns	0.634 ns
2_vs_7	0.820 ***L	0.587 ***L
3_vs_4	**0.858 ***L**	**0.574 ***L**
3_vs_5	0.943 ns	0.728 ns
3_vs_6	0.849 ns	0.550 *L
3_vs_7	0.935 ns	0.710 ns
4_vs_5	**0.900 ***L**	**0.650 ***L**
4_vs_6	**0.960 ns**	**0.780 ns**
4_vs_7	**0.886 ***L**	**0.655 ***L**
5_vs_6	0.890 ns	0.628 ns
5_vs_7	0.897 *L	0.650 **L
6_vs_7	0.877 ns	0.628 ns

Codes: (1) *R. erythrops* n=38, (2) *R. anglocandicans* n=280, (3) *R. laciniatus* n=53, (4) *R. leucostachys* n=92, (5) *R. rubritinctus* n=79, (6) *R. phaeocarpus* n=16 and (7) *R. ulmifolius* n=48.

Significance codes '***' P < 0.0001,'**' P < 0.01,'*' P < 0.05, ns=not significant

L=actual overlap score is less than what is expected by chance

G= actual overlap score is more than what is expected by chance

Paired comparisons with *R. leucostachys* are in bold

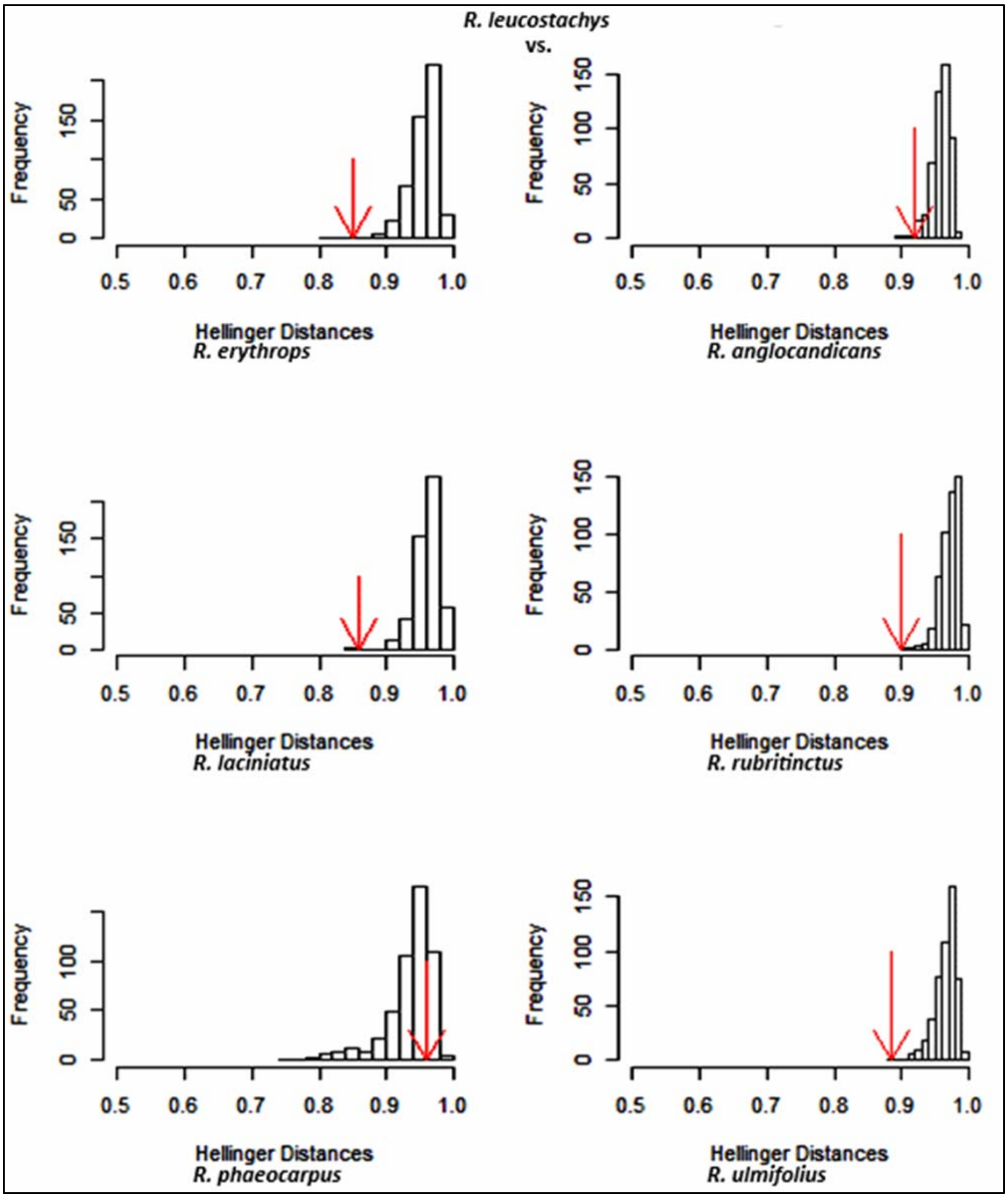

Figure 3.3 Histograms of two-tailed pair-wise comparisons of *R. leucostachys* and six blackberry species occurrence points (niche identity test) using the Hellinger's I_{mod} metric and a null distribution of 500 replicates against the actual overlap scores (arrow). The x-axis represents the similarity value where 0 is no similarity between niches and 1 represents identical similarity of the species pairs

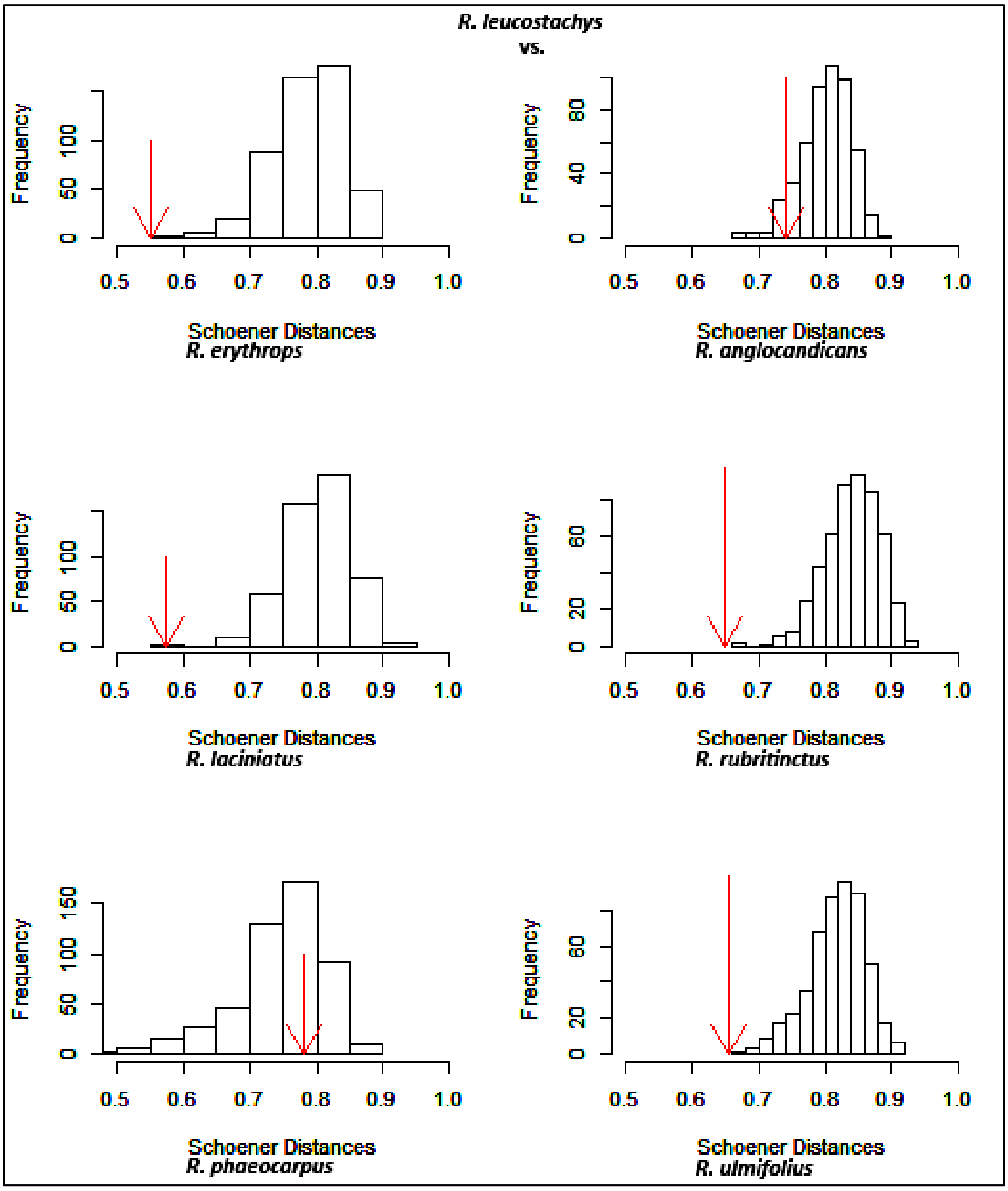

Figure 3.4 Histograms of two-tailed pair-wise comparisons of *R. leucostachys* and six blackberry species occurrence points(niche identity test) using the Schoener's *D* metric and a null distribution of 500 replicates against the actual overlap scores (arrow). The x-axis represents the similarity value where 0 is no similarity between niches and 1 represents identical similarity of the species pairs

Results of the background similarity test

The background similarity test is used to determine whether the ENMs generated are more or less similar based upon the overall region where each species exists, rather than from a randomization of the actual occurrence point data of the species. The general pattern of niche similarity calculated by the background similarity test showed pair-wise overlaps between most blackberry species were typically significantly greater than expected against the random overlap by Hellinger's I_{mod} and Schoener's D statistics (Table 3.2), although comparisons of niche overlap were significantly less than expected by chance for some pairings of blackberry species by Schoener's D statistic (Table 3.2).

Using *R. leucostachys* for illustrative purposes, analysis showed most comparisons of *R. leucostachys* against the other six *Rubus* species were significantly greater (G) than or non-significant than expected from a random overlap by Hellinger's I_{mod} and Schoener's D statistics (Fig 3.5 Table 3.2, Fig 3.5 & 3.6).

The background similarity test appears less biologically meaningful in assessing pair-wise species niche similarity than the niche identity test. This is because the test is based upon randomly positioning individuals of one species within the polygon regions that demarcate the range of both other paired species (see Figure 3.2). However, the polygons contain areas where the bio-climatic conditions are unfavourable for the occurrence of blackberries, leading to an under-estimation of the niche similarity between paired species and thus rendering the test less sensitive.

Table 3.2 Summary table of two-tailed overlap similarity scores between *Rubus* species pairs for the background test. Asterisks indicate significance of randomisation test of the null hypothesis of random overlap

Species pairs	**Hellinger's I_{mod} Distances**	**Schoener's *D* Distances**
1_vs_2	0.820***G	0.535***L
1_vs_3	0.970***G	0.844***G
1_vs_4	0.850ns	0.550***L
1_vs_5	0.907***G	0.660***G
1_vs_6	0.810***G	0.486***G
1_vs_7	0.995***G	0.665***G
2_vs_3	0.820***G	0.535***G
2_vs_4	0.919***G	0.740***L
2_vs_5	0.843***G	0.570***G
2_vs_6	0.869***G	0.634***G
2_vs_7	0.820***L	0.587***L
3_vs_4	0.858***G	0.574ns
3_vs_5	0.943***G	0.728*G
3_vs_6	0.849***G	0.550ns
3_vs_7	0.935***G	0.710***L
4_vs_5	0.900***G	0.650***G
4_vs_6	0.960ns	0.780ns
4_vs_7	0.886ns	0.655**L
5_vs_6	0.890***G	0.628***G
5_vs_7	0.897***G	0.650***G
6_vs_7	0.877***G	0.628***G

Codes: (1) *R. erythrops* n=38, (2) *R. anglocandicans* n=280, (3) *R. laciniatus* n=53, (4) *R. leucostachys* n=92, (5) *R. rubritinctus* n=79, (6) *R. phaeocarpus* n=16 and (7) *R. ulmifolius* n=48.
Significance codes '***' P < 0.0001,'**' P < 0.01,'*' P < 0.05, ns=not significant
L=actual overlap score is less than what is expected by chance
G=actual overlap score is more than what is expected by chance

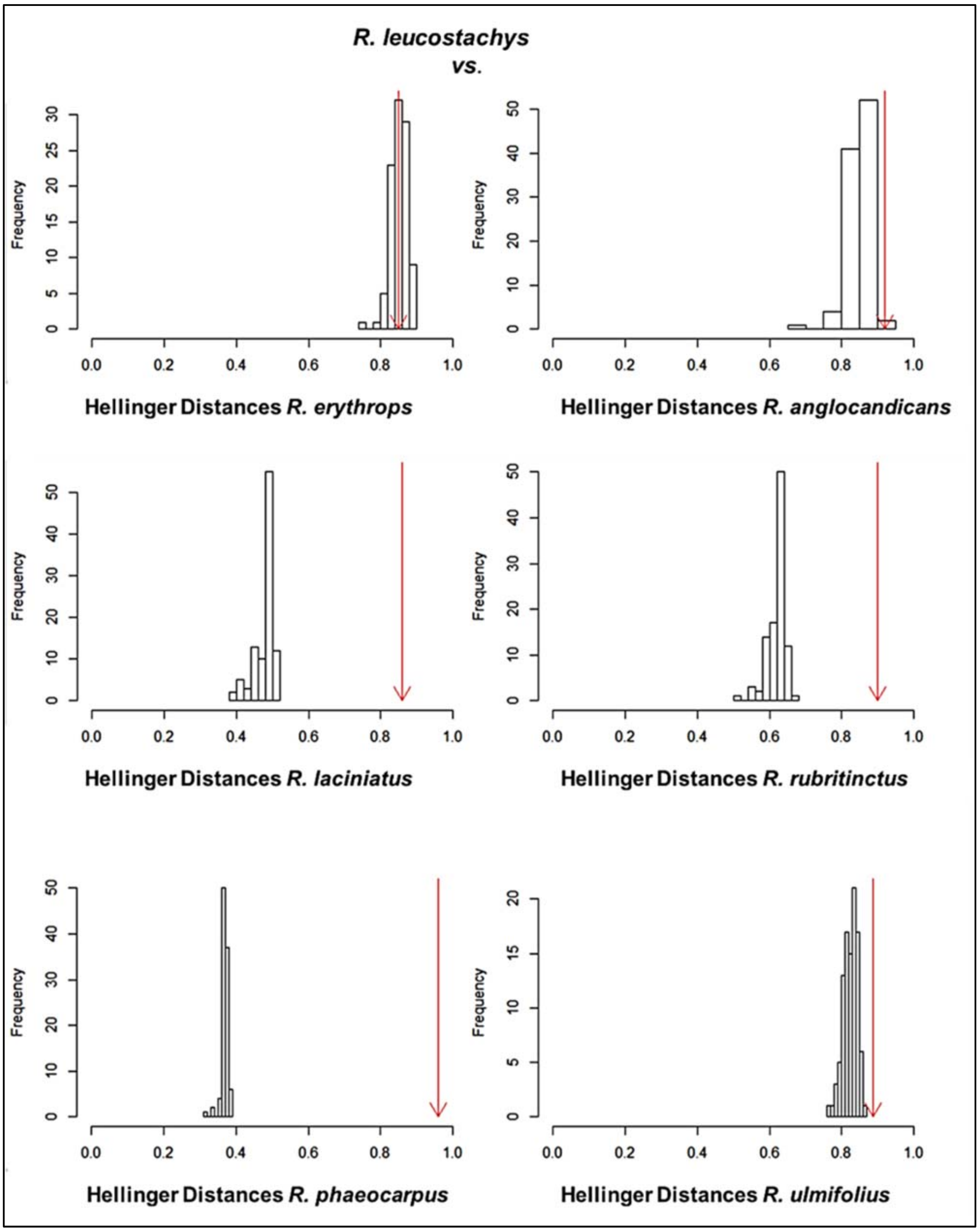

Figure 3.5 Histograms of two-tailed pair-wise comparisons of *R. leucostachys* and six blackberry species background regions occupied using the Hellinger's *Imod* metric and a null distribution of 100 replicates against the actual overlap scores (arrow). The x-axis represents the similarity value where 0 is no similarity between the regions and 1 represents identical similarity of the two species regions

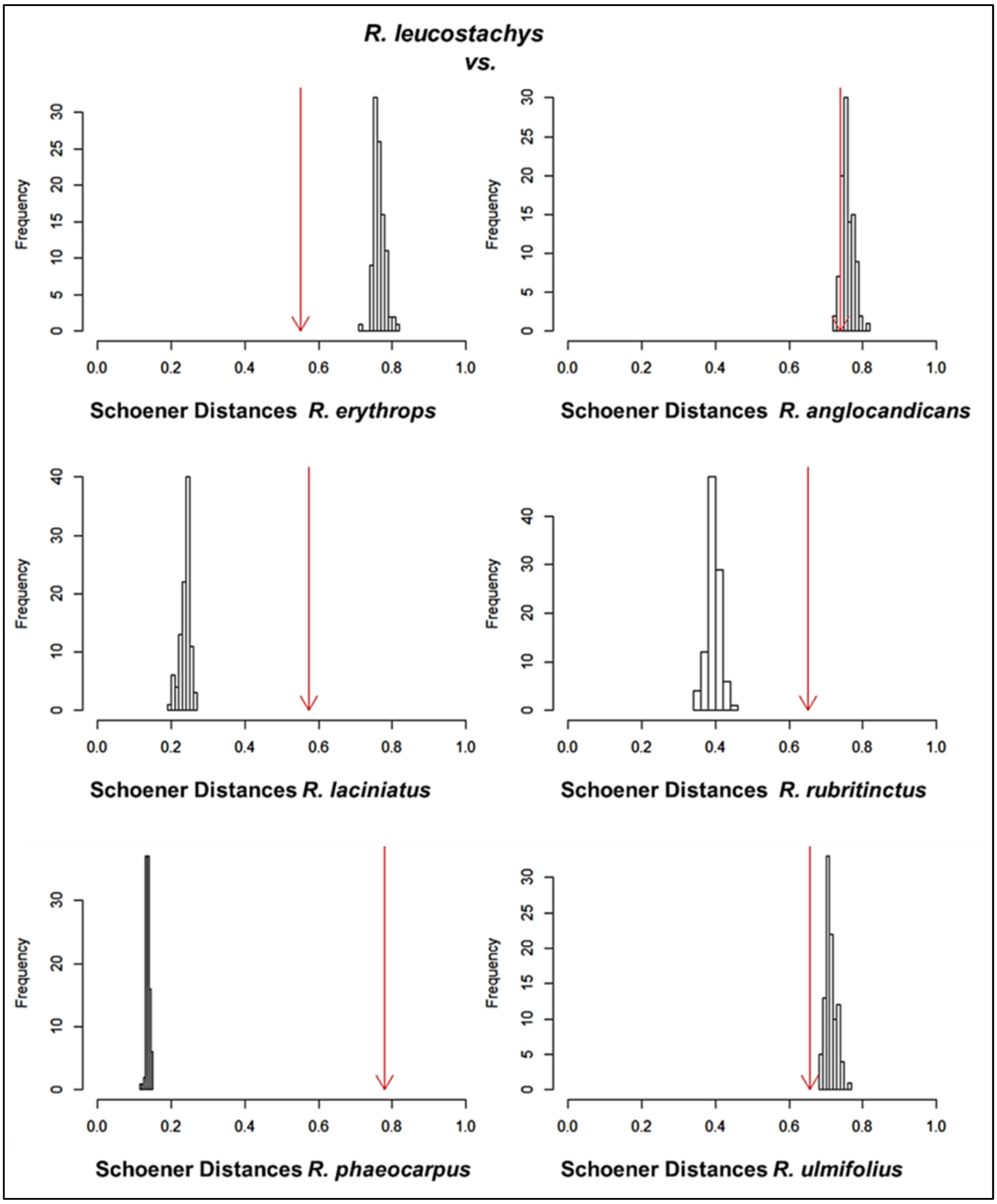

Figure 3.6 Histograms of two-tailed pair-wise comparisons of *R. leucostachys* and six blackberry species background region occupied using the Schoener's *D* metric and a null distribution of 100 replicates against the actual overlap scores (arrow). The x-axis represents the similarity value where 0 is no similarity between the regions and 1 represents identical similarity of the two species regions

Comparing the habitat preferences of Rubus

The multivariate data analysis above of the climatic preferences of *Rubus* species showed significant niche differentiation among blackberry species. To assist in interpreting these patterns, we have compared boxplots of the distribution of environmental variables at the actual localities of individual blackberry taxa with the values of these variables extracted at

500 random locations within the study area. The variables presented are sunlight availability, mean temperature in summer and winter months, winter rainfall, spring evaporation rates and the level of annual aridity where higher numbers represent more humid and wetter conditions. Values vary between species, and it appears that high mean precipitation in July and more mesic conditions are important for the occurrence of blackberry species. The boxplots also suggest *R. erythrops* and *R. laciniatus* may be much less tolerant of dry conditions (Fig 3.7).

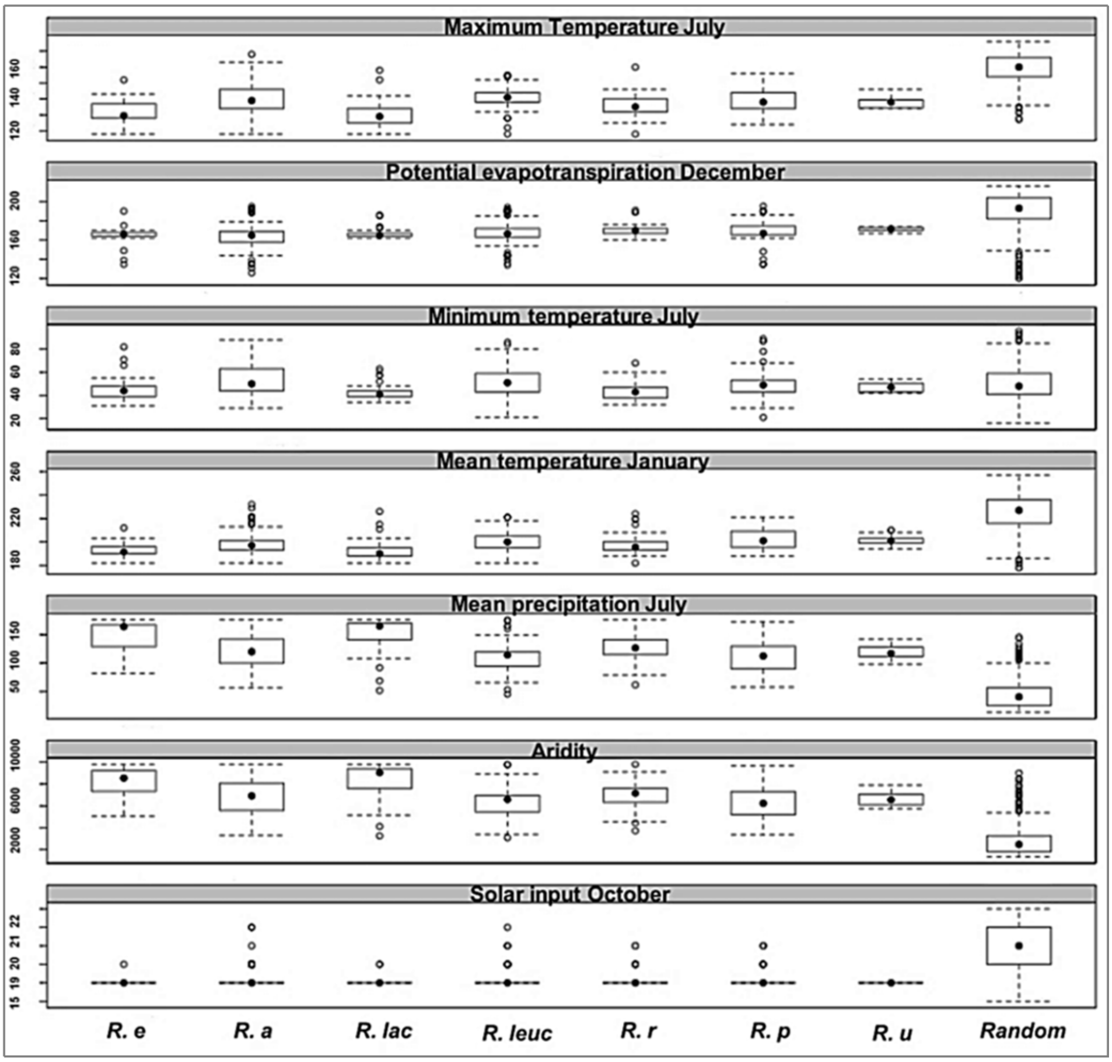

Figure 3.7 Boxplots of the niche occupied by blackberry species in South Australia by seven predictor variables used in building species distribution models for each species against 500 random data points. *R. e =Rubus erythrops* n=51*, R. a =Rubus anglocandicans* n=280 *R. lac=Rubus laciniatus* n=53*, R. leuc=Rubus leucostachys* n=92*, R. r=Rubus rubritinctus* n=79*, R. p=Rubus phaeocarpus* n=10*, R. u=Rubus ulmifolius* n=48. Temperature (maximum, mimimum and mean) in º C; potential evapotranspiration and mean precipitation in mm; Aridity is dimensionless and solar input is measured in MJ.

By comparison, the data for *R. leucostachys, R. anglocandicans* and *R. rubritinctus* suggested these species may be more tolerant of periods of aridity but considerable overlap exists between all blackberry species (Fig 3.7).

The boxplots of *R. ulmifolius* for all variables suggest this species may occupy areas with low variability in moisture, aridity and temperature (Fig 3.7).

Discussion

Differences in species niches

Multivariate analysis of the climatic variables used to build species distribution models identified numerous significant differences between individual species, as judged by the niche identity test. Although *R. fruticosus* agg. taxa occur in populations that sometimes overlap spatially in the Mt. Lofty Ranges, it is apparent that some taxa are significantly differentiated and occupy differing climatic niches within the region.

The background test used to define the niches of *R. fruticosus* agg. species appeared to be less informative and biologically meaningful because the polygonal representation of the range of each species derived from GIS data appeared to include areas where blackberries are not likely to occur due to poor climatic conditions.

Species distribution modelling of invasive blackberry taxa in Chapter 2 showed that *R. anglocandicans* and *R. leucostachys* are potentially capable of occupying extensive additional areas throughout southern Australia although the species distribution models are a static representation of the species' fundamental niches. The niche differences calculated by the niche identity test showed *R. anglocandicans* and *R. leucostachys* are significantly differentiated with respect to their respective bioclimatic niches.

Rubus fruticosus agg. species are closely related and are often assumed to occupy similar habitats and niches (Evans *et al.*, 2007). Niche statistics calculated by ENMTools quantitatively separated the *R. fruticosus* agg. species niches in a complex landscape where some populations of species sometimes co-occur, providing a much better understanding of the niche characteristics preferred by individual species.

The data presented in Chapter 2 & 3 for *R. ulmifolius*, which is the only diploid taxa in the group, supports the assumption it is an adaptable and widespread species in the Australian landscape compared to the putative polyploid taxa *R. laciniatus, R. erythrops,* and *R. rubritinctus.* Nevertheless, the narrow bio-climatic tolerances demonstrated by *R. ulmifolius* in the multivariate analysis also suggest that while it is a substantial invader, by the area in South Australia that it currently occupies, it is more narrow in its ecological preference than other *R. fruticosus* agg. taxa and potentially less capable of tolerating large climatic shifts.

Linking niche differentiation to invasiveness in new ranges

The patterns of niche similarity calculated using ENMTools suggests the calculated niches of some *R. fruticosus* agg. species display a pattern of differentiation.

Rubus anglocandicans is significantly differentiated in the landscape and less similar in its niche compared with most other blackberry taxa (Table 3.1) which supports the assumption it is tolerant of a wide range of bio-climatic conditions (Evans & Weber, 2003).

Some alien plant taxa in Australia where comparisons can be made between a plant's habitat preferences in their introduced range and their native range provide some evidence to demonstrate invaders frequently occur in novel niches in climatic conditions outside those occupied in their native range (Gallagher *et al.*, 2010; Wilson *et al.*, 2011; Beaumont *et al.*, 2014). For example, Gallagher *et al.* (2010) found that of the 26 minor weed taxa introduced into Australia, the majority of species (19) exhibited a shift in their climatic and biotic range tolerances when matched to their native ranges and concluded that the species were able to occupy broader niches (as invaders) than in their native range.

Where native range data are not available for invasive taxa, measuring niche similarity between species provides a method of statistically comparing the niches occupied by related species and comparing the niche breadths of species with differing levels of invasiveness.

In our study, the measurement of niche similarities and differences between paired species may partially explain some of the underlying factors that influence the extent of ranges for some invasive blackberries in South Australia since introduction (Giles & Pascoe, 1868). Today, the two most commonly found species, *R. anglocandicans* and *R. leucostachys,* occupy the largest ranges (similar in extent) within the landscape but they are statistically dissimilar in their niche preferences measured by niche identity and not likely to occupy the same habitats.

Extending SDM with environmental niche modelling

Extending the understanding of an invasive species' potential range expansion using SDM and environmental niche modelling (ENM) provided a more complete understanding based upon the more detailed individual bio-climatic preferences that may be particularly important where related species overlap in their invasive ranges. While species distribution modelling of blackberry taxa (in Chapter 2) was informative and mapped differences in the potential expansion of each blackberry species in geographic space based upon a suite of bio-climatic variables, environmental niche modelling provided statistical comparisons of the similarity of each blackberry species' niche based upon the underlying MAXENT model.

While this study is limited, it represents a basic introduction into ways to extend species distribution modelling that allows testing of the niche similarity of individual blackberry species. However, considerable new work is needed to include native range data and enhance the understanding of the niche preference of individual taxa to develop a broader consensus in species-level quantitative ecological biogeography of invasive blackberries within Australia.

Concluding Remarks

The metrics of niche similarity (EMNTools) further quantify the differences in the niches of invasive blackberry taxa in Australia and extend the interpretative power of species distribution modelling. Using niche identity and niche background tests to assess and quantify related species niche differences assists in interpreting the outcomes of static species distribution models and in forecasting the anticipated course of invasion of different species.

The question of whether any species have undergone a shift in climate tolerances in their introduced ranges cannot be properly assessed without native range data for comparison. Such data would be valuable in assessing the adaptability of species in their new niches and in further interpreting the outcomes of environmental niche models. This aspect of predictive assessment of the potential of invasive blackberry species would be a valuable asset in biodiversity management.

Acknowledgements

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Appendix (App.) Chapter 3

Summary histograms for niche identity

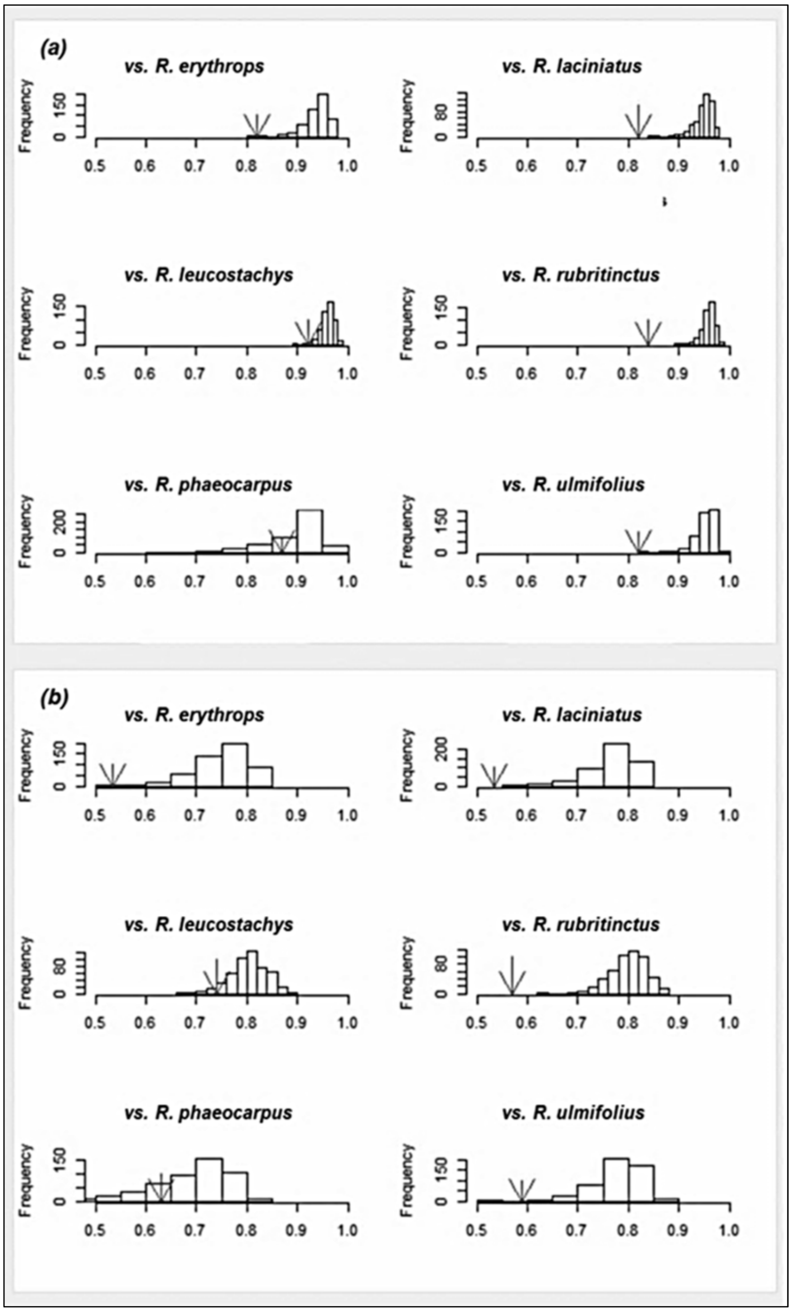

Figure A3.1 Niche identity test histograms comparing the pairings of *R. anglocandicans* vs. other species, the niche overlap values (arrow) are compared to the null distribution with two metrics. Paired comparisons are significantly differentiated in Fig. A3.1, Hellinger's I_{mod} and Schoener's D suggest the defined niche is more dis-similar between and *R. anglocandicans* and other taxa. The niche appears more similar between *R. anglocandicans* and *R. leucostachys* but it is not identical. The X-axis is the similarity values of 0-1 for each species pair.

(a) = Hellinger's I_{mod}
(b) = Schoener's D

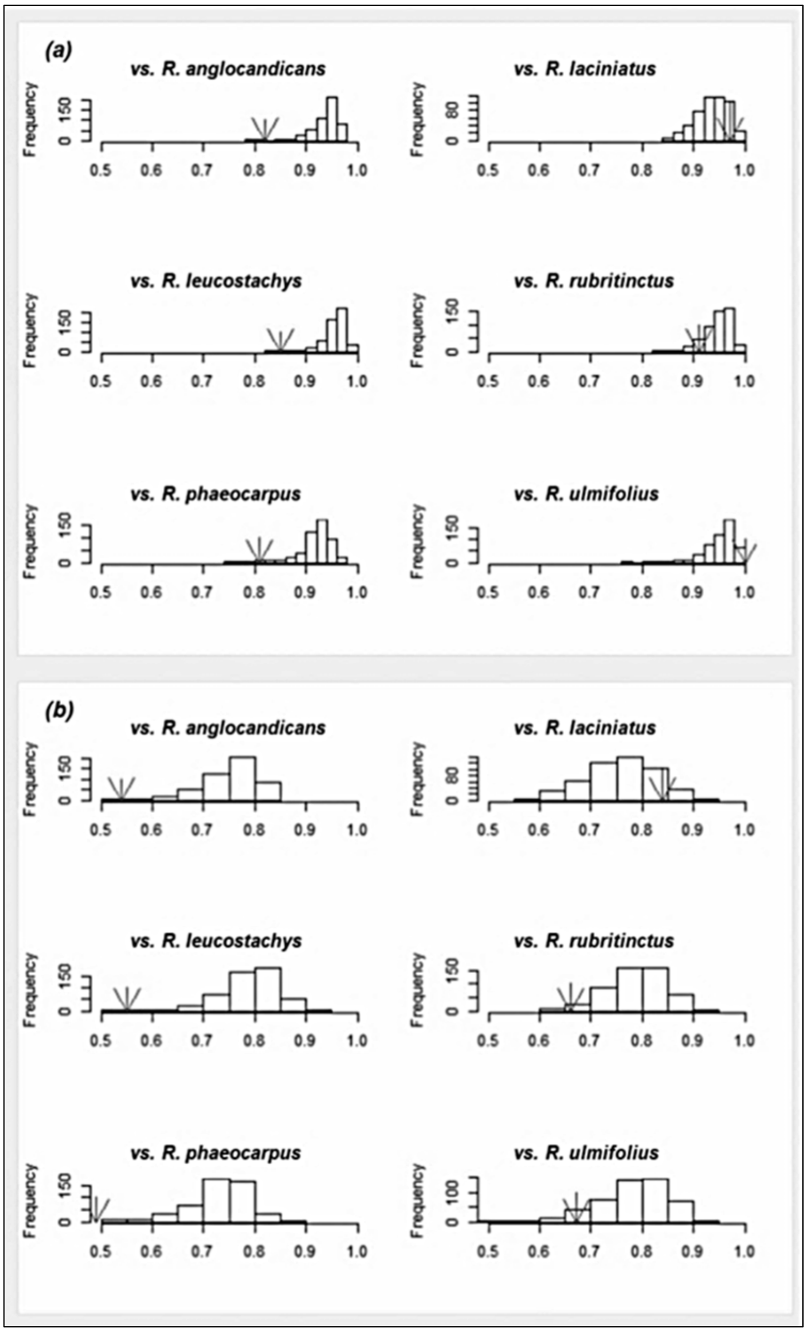

Figure A3.2 Niche identity test histograms comparing the pairings of *R. erythrops* vs. other species, the niche overlap values (arrow) are compared to the null distribution with two metrics. Hellinger's I_{mod} and Schoener's *D* suggest the defined niche is more dissimilar between *R. erythrops* vs. *R. anglocandicans* and *R. leucostachys* and are more similar to *R. rubritinctus* and *R. ulmifolius.* The X-axis is the similarity values of 0-1 for each species pair.
(a) = Hellinger's I_{mod}
(b) = Schoener's *D*

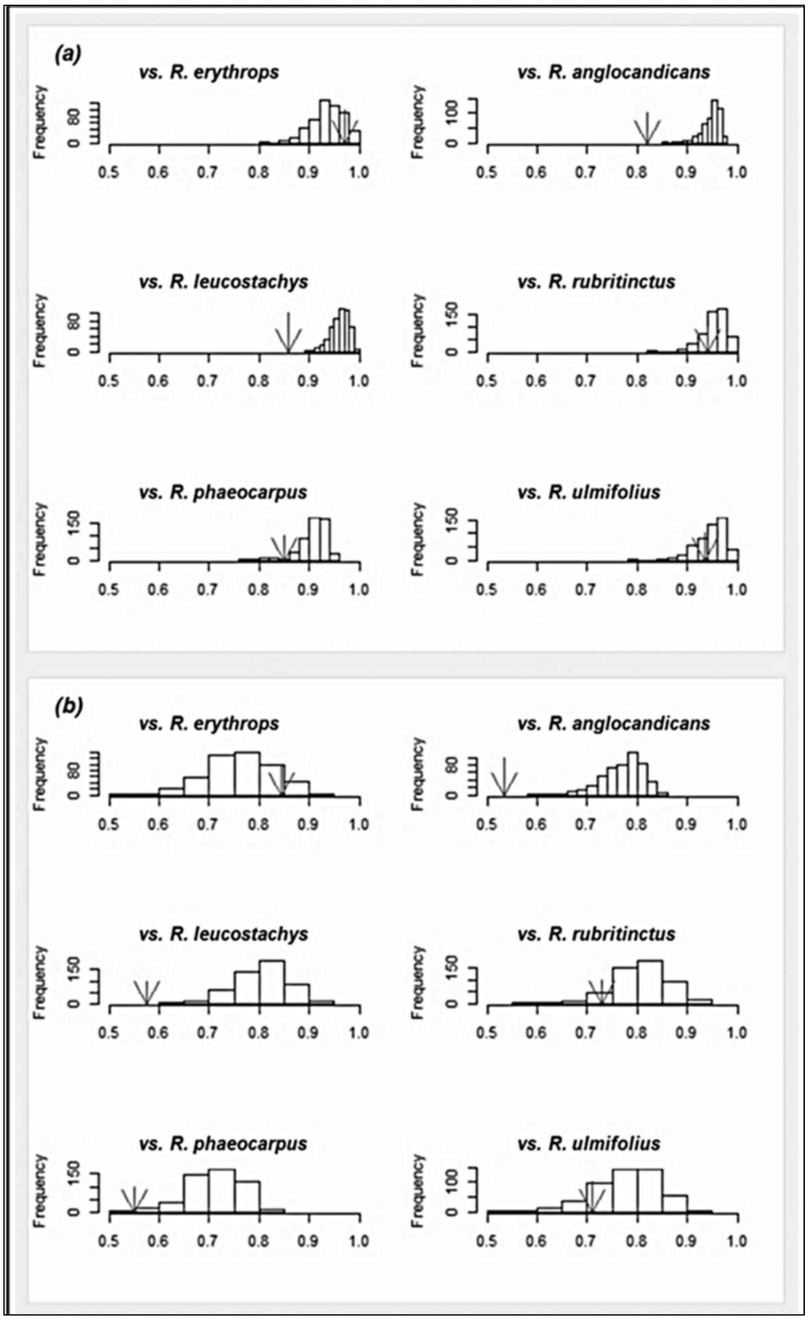

Figure A3.3 Niche identity test histograms comparing the pairings of *R. laciniatus* vs. other species, the niche overlap values (arrow) are compared to the null distribution with two metrics. Hellinger's I_{mod} and Schoener's D suggest the defined niche is most dissimilar between *R. laciniatus* vs. *R. anglocandicans* and *R. leucostachys*. The X-axis is the similarity values of 0-1 for each species pair.
(a) = Hellinger's I_{mod}
(b) = Schoener's D

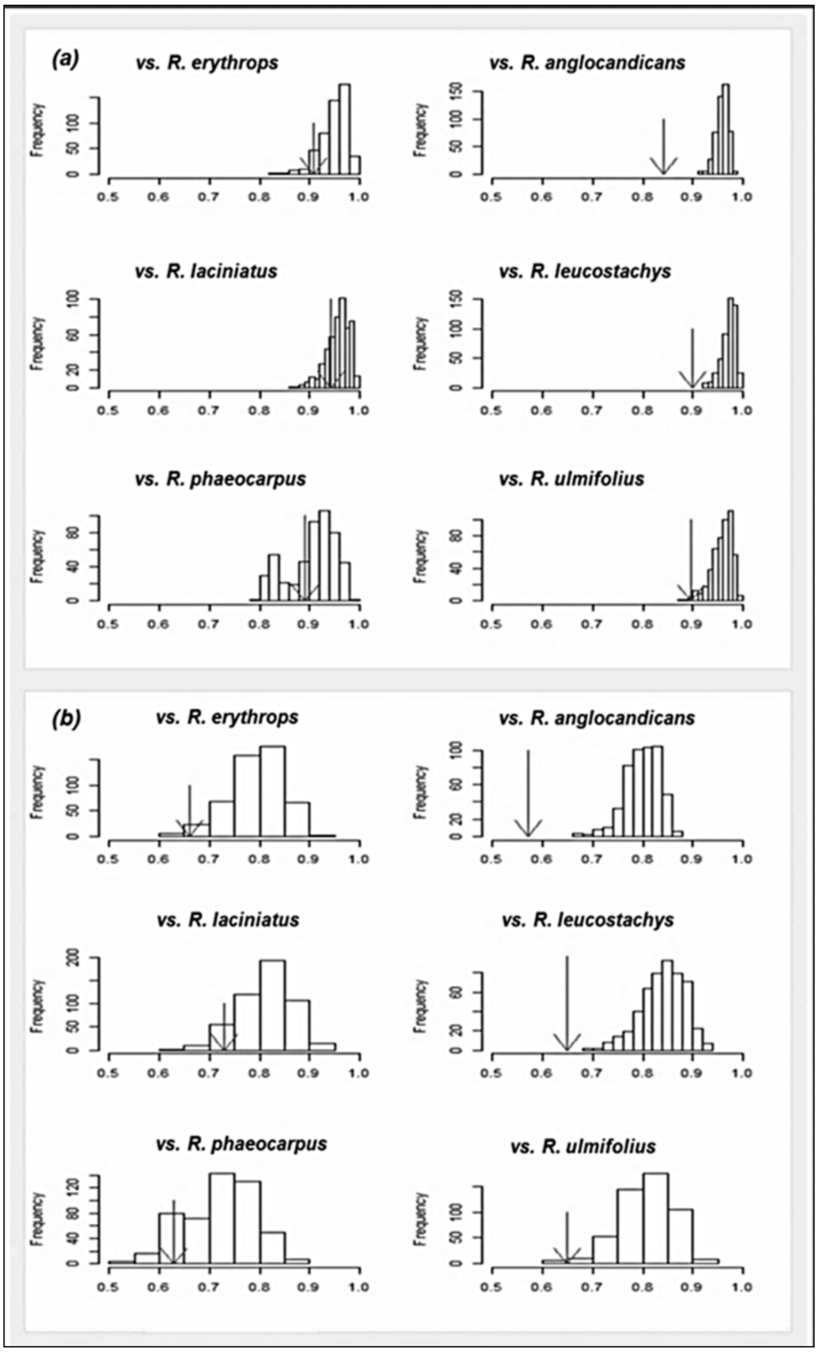

Figure A3.4 Niche identity test histograms comparing the pairings of *R. rubritinctus* vs. other species, the niche overlap values (arrow) are compared to the null distribution with two metrics. All paired comparisons are significantly differentiated, except *R. phaeocarpus.* All metrics suggest the defined niche is more similar between *R. rubritinctus* and *R. laciniatus* and most dissimilar between *R. rubritinctus* and *R. anglocandicans and R. leucostachys.* The X-axis is the similarity values of 0-1 for each species pair.
(a) = Hellinger's I_{mod}
(b) = Schoener's D

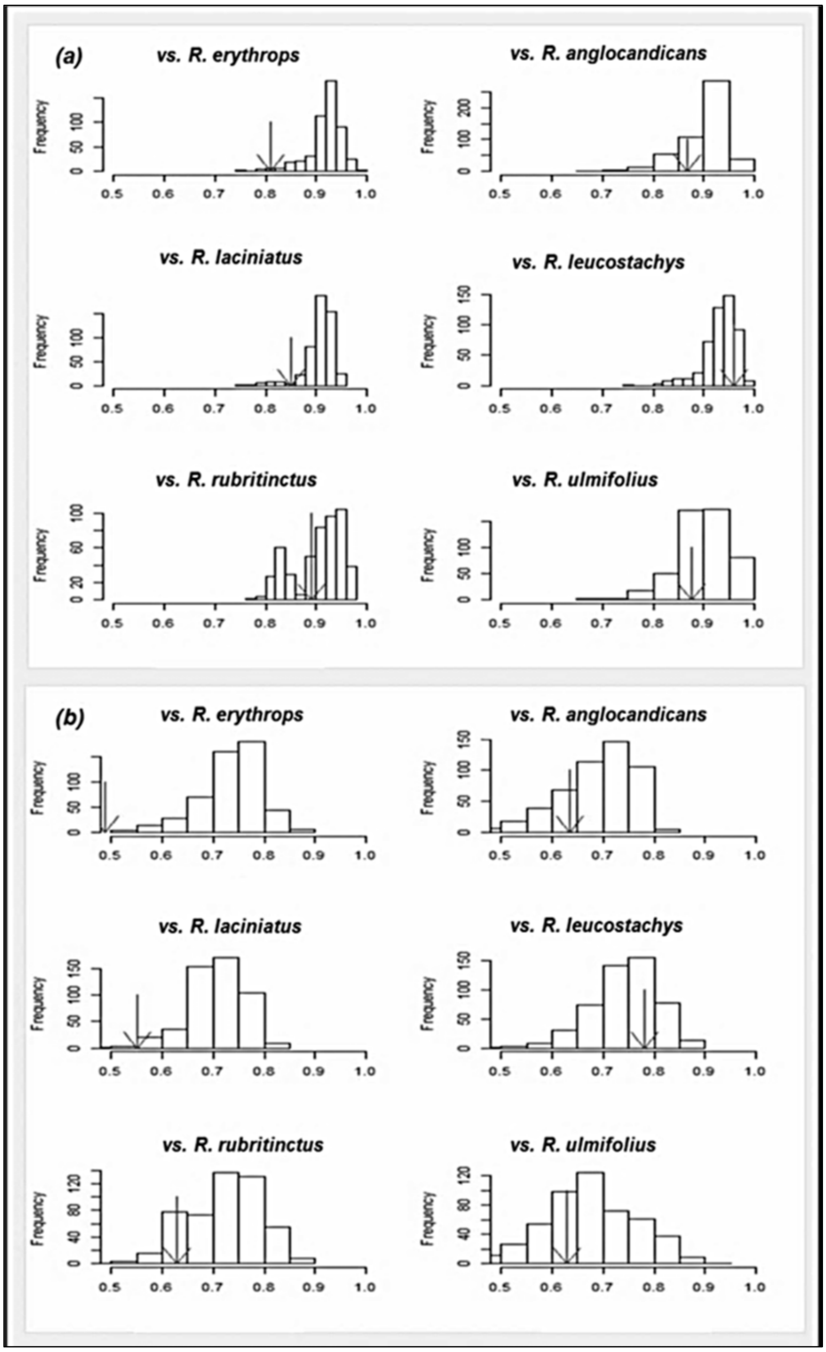

Figure A3.5 Niche identity test histograms comparing the pairings of *R. phaeocarpus* vs. other species, the niche overlap values (arrow) are compared to the null distribution with two metrics. Only paired comparisons between *R. rubritinctus* and *R. erythrops* and *R. laciniatus* are significantly differentiated by metrics *(a)* and *(b).* These metrics suggest the defined niche is more similar between *R. phaeocarpus* and *R. laciniatus* and most dissimilar between *R. phaeocarpus* and *R. erythrops.* The X-axis is the similarity values of 0-1 for each species pair.
(a) = Hellinger's I_{mod}
(b) = Schoener's *D*

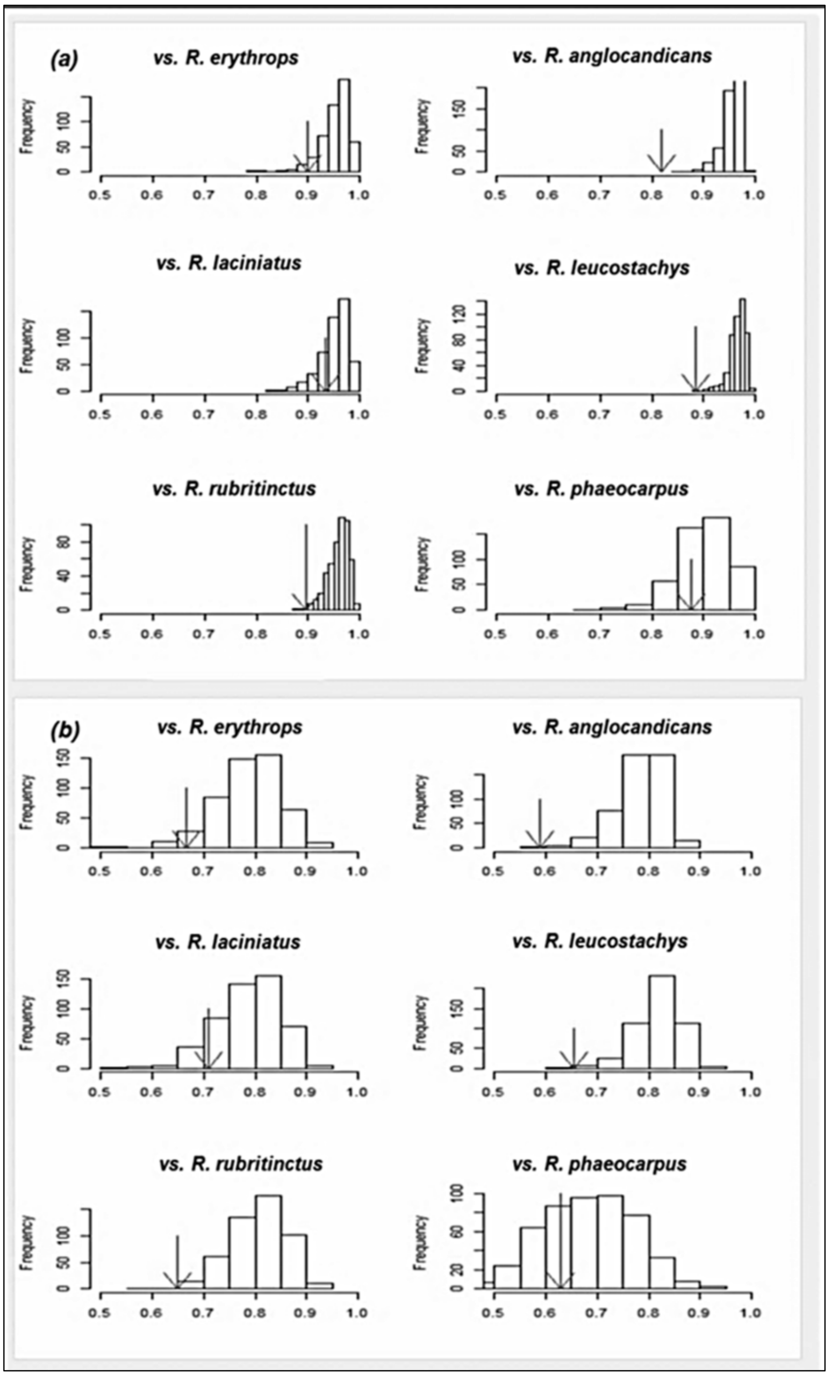

Figure A3.6 Niche identity test histograms comparing the pairings of *R. ulmifolius* vs. other species, the niche overlap values (arrow) are compared to the null distribution with two metrics. Most paired comparisons are significantly differentiated, except *R. phaeocarpus.* Metrics suggest the defined niche is more similar between *R. ulmifolius* and *R. laciniatus* and most dissimilar between *R. ulmifolius* and *R. anglocandicans* and *R. leucostachys*. The X-axis is the similarity values of 0-1 for each species pair.
(a) = Hellinger's I_{mod}
(b) = Schoener's *D*

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Chapter 4

Modelling the biotic and abiotic preferences of blackberry invading native vegetation in the Mt. Lofty Ranges

[1]John Marshall, [2] Molly A. Whalen, [3] Duncan A. Mackay

[1, 2, 3] Flinders University, School of Biological Sciences, GPO Box 2100, Adelaide, S.A. 5001, Australia.

Abstract

Aim European blackberries are an important Weed of National Significance (WONS) in Australia. This study was undertaken to develop a greater knowledge of the underlying biology, ecology and niche preferences of individual species of the *Rubus fruticosus* L. aggregate that invade native plant communities.

Location Mt. Lofty Ranges, South Australia, Australia

Methods We surveyed the presence and absence of ten blackberry species within randomly selected plots in areas of native vegetation in the Mt. Lofty Ranges region and developed explanatory models to predict the occurrence of individual species.

Results Logistic regression analyses (GLMM) of relationships between measured ecological and biological attributes of survey sites where taxa of the *R. fruticosus* agg. occurred show that the presence of different species varied as a function of different combinations of variables characteristic to each species. *Rubus anglocandicans* occurred over a broad range of aridity but avoided areas of high sunlight intensity. The presence of *R. erythrops* appeared partly dependent on gaps in native vegetation. *Rubus leucostachys* appeared to have a very low association with native vegetation in the initial survey.

Main Conclusions Results from the analysis of our survey demonstrated that different blackberry species invade native vegetation communities differently and their distribution throughout the Mt. Lofty Ranges was influenced by the climatic tolerances and discrete biotic attributes of each species.

Our survey of factors affecting the presence of *R. fruticosus* agg. species in native vegetation highlighted the variability of each species in association with native vegetation communities.

Keywords ***Rubus fruticosus*, blackberries, processes of invasion, weed of national significance, Mt. Lofty Ranges, edge effect**

Introduction

The attributes that make weeds successful invaders in new ranges are diverse and have been significantly associated with climate suitability, tolerance to greater climatic regimes, adaptation, a diversity of reproductive behaviours and competition (Hayes & Barry, 2008). However, a systematic understanding of the attributes that assist exotic plant taxa in becoming successful invaders is still incomplete particularly within Australia (French *et al.*, 2014). Like all plant taxa, the biotic and abiotic drivers that control and influence where invasive plant species are able to exist is strongly determined by a fundamental ability to adapt in new ranges (Richardson & Pyšek, 2006).

In our study, we identified the biotic, abiotic and landscape preferences of individual species of the invasive *Rubus fruticosus* L. aggregate. These taxa have invaded large ranges across Australia where they have become problematic weeds. Although some research has focused on finding solutions to the agricultural threat they pose (as a group), a detailed understanding of the biological and ecological attributes of individual species that aid them in invasion is still lacking (French *et al.*, 2014).

Invasive blackberry species introduced into Australia since European settlement are an ongoing problem not only because of the threat they pose to agriculture but also because of the significant changes to native vegetation communities that they induce, making management of these taxa more problematic (Davies, 1998; French, 2012; Mason *et al.*, 2012).

Background to the Rubus fruticosus *L. agg.*

European blackberries are natives of Britain and Europe (Edees & Newton, 1988). The taxa of the *R. fruticosus* agg. have a diversity of reproductive mechanisms where most species of the aggregate share a clonal breeding system that can generate fecund seed on the maternal line without gamete reduction, known as pseudogamous apomixis (Edees & Newton, 1988; Amor *et al.*, 1998). The dominant mode of reproduction in natural and agricultural systems though is likely to be through multiple methods of vegetative reproduction, both from the development of underground suckers from the root system that form into new daughter plants as well as layering of aboveground primocanes, and through a method of tip rooting of primocanes apices in the autumn and winter (Watson, 1958; Amor, 1974). Vegetative spread is characterised by a radial expansion pattern of primocanes, allowing a rapid colonization of suitable habitats particularly in woodland margins, along streamlines and in forest gaps (Watson, 1958; Amor, 1974; Taylor, 2005).

The diversity of habitats of the **R. fruticosus** *agg.*

Rubus fruticosus agg. belongs to subgenus *Rubus* of the genus *Rubus* L. (Rosaceae) which occupies a diversity of habitats worldwide from the edge of dry deserts to the Arctic Circle (Clark *et al.*, 2007), but very few studies have quantified specific ecological preferences of individual species in both their native and invasive ranges. Most studies of individual taxa over the last three hundred years have centred in Britain and Europe, concentrating on the challenging morphology of *Rubus* subgenus *Rubus* but also often describing the marked soil and habitat preferences of some important species (Edees & Newton, 1988). Gazda and Janas (2011) provided a quantitative profile of the light tolerances and the variation in habitats preferred by *Rubus hirtis* (Waldst. & Kitt.) in a series of studies of one of the most common members of the *R. fruticosus* agg. in eastern Europe (Gazda *et al.*, 2007; Gazda & Kochmanska-Bednarz, 2010). One of the most detailed studies of a commonly found species in its native range placed *Rubus vestitus* Weihe in its ecological context in both Great Britain and Europe where it prefers calciferous soils (Taylor, 2005).

As an invasive taxon, *R. fruticosus* agg. occupies a diversity of habitats in its invasive ranges in the north-western United States and in Australia. Some studies have investigated its overall tolerance to drought, shade and soil conditions (Amor, 1973; Lindenmayer & McCarthy, 2001; McDowell, 2002; Caplan & Yeakley, 2006) but no consolidated understanding of the ecological preferences of individual species of the *R. fruticosus* agg. in its invasive range is available, particularly for the species found in Australia.

Ecology and biology of blackberries in Australia

The majority of the ecological information on the invasion processes of plants of the *R. fruticosus* L. aggregate in the Australian context has been generated principally from trying to understand the processes of invasion in commercial forestry plantations in southern Australia (Amor & Miles, 1974; Amor *et al.*, 1998; Lindenmayer & McCarthy, 2001).

Amor's early work (Amor, 1973, 1974; Amor & Miles, 1974) formed the basis of the earlier understanding of the ecology and biology of the blackberry species, *Rubus procerus* P. J. Muell. (now *Rubus anglocandicans* A. Newton). Lindenmayer and McCarthy (2001) quantified the spatial distribution and some ecological associations with native vegetation of plants in the *R. fruticosus* agg. near *Pinus radiata* D. Don plantations in southern New South Wales. They found significant patterns in the occurrences of blackberries in eucalypt forest remnants in the proximity of *P. radiata* plantations. Native vegetation survey plots located close to the surrounding pine plantations were more susceptible to invasion by *R. fruticosus* agg. than more distant plots, indicating that edge effects and disturbance levels were important correlates of invasion.

More generally, the invasion by blackberries has often characterized them as occupying disturbed land and invading at the margins (Edees & Newton, 1988), even though descriptions have often been based upon the ecological behavior of *R. fruticosus* agg. in their native ranges in Europe and Britain or upon very limited studies in the Australian context, which do not sufficiently explain the ecological and biological reasons for the success of blackberries in introduced ranges (Watson, 1958; Edees & Newton, 1988; Amor *et al.*, 1998). Watson (1958) proposed that phenotypic plasticity of individual species in introduced ranges, with particular reference to invasion within Australia of one species, *R. procerus* (now *R. anglocandicans*), was helpful in partly explaining their invasion success, but his hypothesis remains untested.

A major impediment to expanding the understanding of the invasion biology of blackberries in Australia is that the *R. fruticosus* agg. has usually been treated as a single entity and hence variation among species within the aggregate regarding their habitat preferences and potential for invading native vegetation has been largely overlooked. Another element that has made research into the specific ecological differences between species of the aggregate problematic and difficult to interpret is the lack of taxonomic understanding as to the number and identification of Australian exotic blackberries. However, a taxonomic study of Australian taxa (Evans *et al.*, 2007) has helped to resolve the uncertainties regarding the number of introduced species within Australia. Another important advance is the development of an interactive taxonomic key, which has aided in the identification of individual taxa (Barker & Barker, 2005). In Australia, there have been very limited empirical studies relating to the breeding system, biology and spatial ecology of *R. fruticosus* agg. and in particular, as to how individual species of the *R. fruticosus* agg. invade and degrade natural systems (Adair & Bruzzese, 2006). Broadening the understanding of the mechanisms of invasion and the ecological preferences of individual species of the *R. fruticosus* agg. in Australia is important for their control, as species of this group are likely to differ in their ecological requirements and invasiveness and therefore respond differently to particular control measures (pers. comm. Philip Cramond, Bio-Control Officer, Natural Resource Management (NRM) (Mt. Lofty). Our principal hypothesis is that individual taxa of the aggregate will invade native vegetation differently due to the varying biotic and abiotic preferences of each individual species. We expect these differences to be asymmetric among species and this may help to explain why some species appear more invasive in Australia and South Australia than others.

Introduction of blackberries into South Australia

Of the fourteen putatively facultatively apomictic species and the one sexual species (*Rubus ulmifolius* Schott) found in Australia, ten species occur in South Australia (Evans *et al.*, 2007). Blackberries are thought to have originated from Europe and Britain and to have been introduced into Australia in the 1800s by colonial settlers for use in horticulture (Giles & Pascoe, 1868).

Methods

GIS methods

Mapping surfaces were sourced from a variety of providers including the School of Geography, Population and Environmental Management, Faculty of Science and Engineering, Flinders University, the Department of Environment Water and Natural Resources (DEWNR) and Geoscience Australia. We obtained the global aridity index (AI) layer from the Consultative Group on International Agricultural Research (CGIAR) Consortium for Spatial Information (2012). This index is calculated as the ratio of average annual precipitation to potential evaporation over a 50-year period (1950-2000) and higher values of aridity index represent more humid and wetter conditions. The digital elevation model (DEM) is a one second derived surface from satellite data (Gallant *et al.*, 2011). The GIS mapping surfaces used for preparation of data for field surveys and statistical analysis were re-projected as required using the Universal Transverse Mercator (UTM) and Geographic Coordinate System (GCS). GIS surfaces in polygon format were converted to prediction surface raster maps of AI and DEM to allow extraction of raster point values of blackberry occurrences for statistical analysis of survey data.

Field survey locations

Over the period June 2011 to June 2012, we collected data on the presence and absence of blackberry species from field surveys of randomly placed plots in thirteen remnant vegetation sites covering approximately 3000 sq. km in the Mt. Lofty Ranges (MLR) (Fig. 4.1). These sites are geographically and environmentally representative of the Mt. Lofty region classified by the Köppen-Geiger climate classification (Peel *et al.*, 2007).

A diversity of vegetation communities exists within the Mt. Lofty Ranges region including sclerophyll forests, drier heathy woodlands, grassy woodlands, grasslands, mallee, swamps, wetlands and various coastal and estuarine ecosystems (Department for Environment and Heritage 2009). The undisturbed native vegetation sites were located mostly in conservation parks, which varied in size between 2.5 and 5 sq. km within the Mt. Lofty Ranges of South Australia between latitudes -34.300000, -35.500000 dd and longitudes 138.500000, 139.300000 dd (Fig 4.2).

Development of random sampling quadrats

We selected random quadrats using GIS by projecting a grid of 100m^2 over the whole survey area using the "fishnet tool" in ArcMap® (ESRI®, 2010, 2012) and then randomised the selection of unique quadrats within each of the sites to generate 34-38 100m^2 sampling quadrats at each site (Fig. 4.1& Fig. 4.2). There was a total of 463 quadrats across all 13 sites. We then GPS tagged the corner of each quadrat and uploaded the data to a Garmin GPS, for location finding in the field (Fig. 4.2).

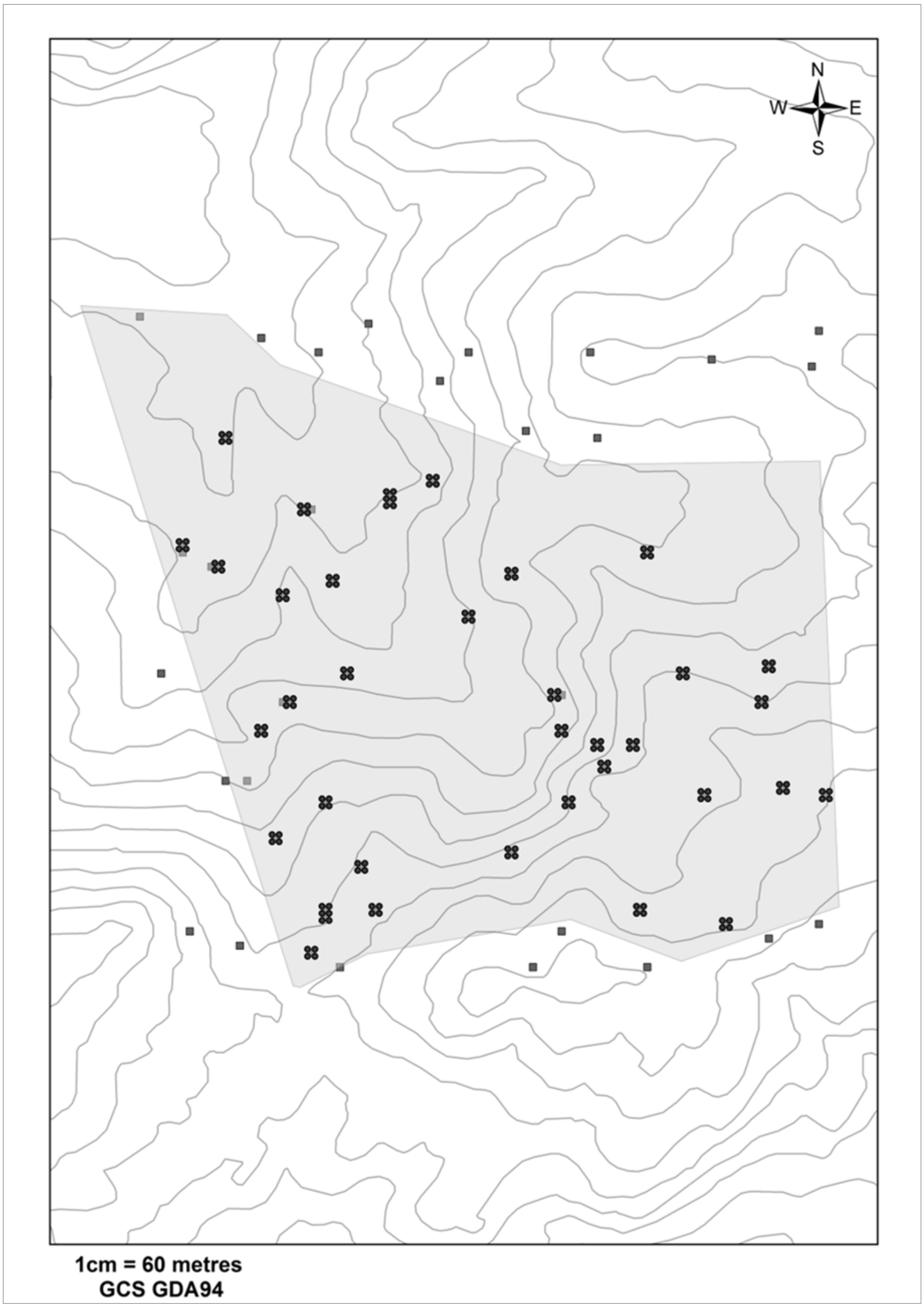

Figure 4.1 Example of the random sampling grids , generated in ArcMap® and projected over the Charleston Conservation Park (grey) with 50m contours, the map elements were sourced from Department of Environment Water and Natural Resources (2010a, 2010b)

We used a modified point quarter survey in the study for collection of the vegetation metrics (Brower *et al.*, 1990), (Table 4.1). At each quadrat, we measured vegetation structure above 5 meter and 0.1-5 meter (excluding grasses) from the centre point of each quadrat and recorded the distance to the closest plant using a 5m survey pole and a Nikon forestry lazer (starting in the NE section and moving clockwise). We also measured trunk diameter and canopy spread of native plants adjacent to and within each quadrat in this manner (Table 4.1).

Table 4.1 Table of data collected from each quadrat at the field sites

Biotic data	**Abiotic data**
Presence/absence of five blackberry species	Percent soil moisture content (mean of three readings)
Presence/absence of Scotch broom, *Cytisus scoparius* (L.) Link, and African daisy, *Senecio pterophorus* DC.	Intensity under-canopy sunlight (Lumens) (mean of five readings)
Density of five blackberry species	Percent cover of rock
Density of broom and African Daisy taxa	Percent cover of bare ground
Closest tree above 5 meter in height from quadrat centre • height (m) • trunk diameter at one meter (m) • distance from quadrat centre (m) • canopy width (m)	Slope across quadrat measured in degrees
Closest scrub 0.1-5 meter in height from quadrat centre • height (m) • distance from quadrat centre (m) • canopy width (m)	

Collection of field data

We collected data from each quadrat twice, once in winter /spring and once in summer /autumn to assess seasonal changes. In addition, we divided the 100m^2 quadrats into four 25m^2 sub-quadrats and surveyed these in a clockwise fashion for presence/absence of weed taxa and abiotic data starting in the north-west quadrat. We constructed a standardised scale to represent the density of blackberries as sparse, moderate or abundant (App., Fig. A4.1).

Identification of blackberry taxa

Blackberry taxa were identified using the interactive key "An identification tool to introduced and native *Rubus* in Australia" (Barker & Barker, 2005), and new material was vouchered and included in the *Rubus* collection (AD).

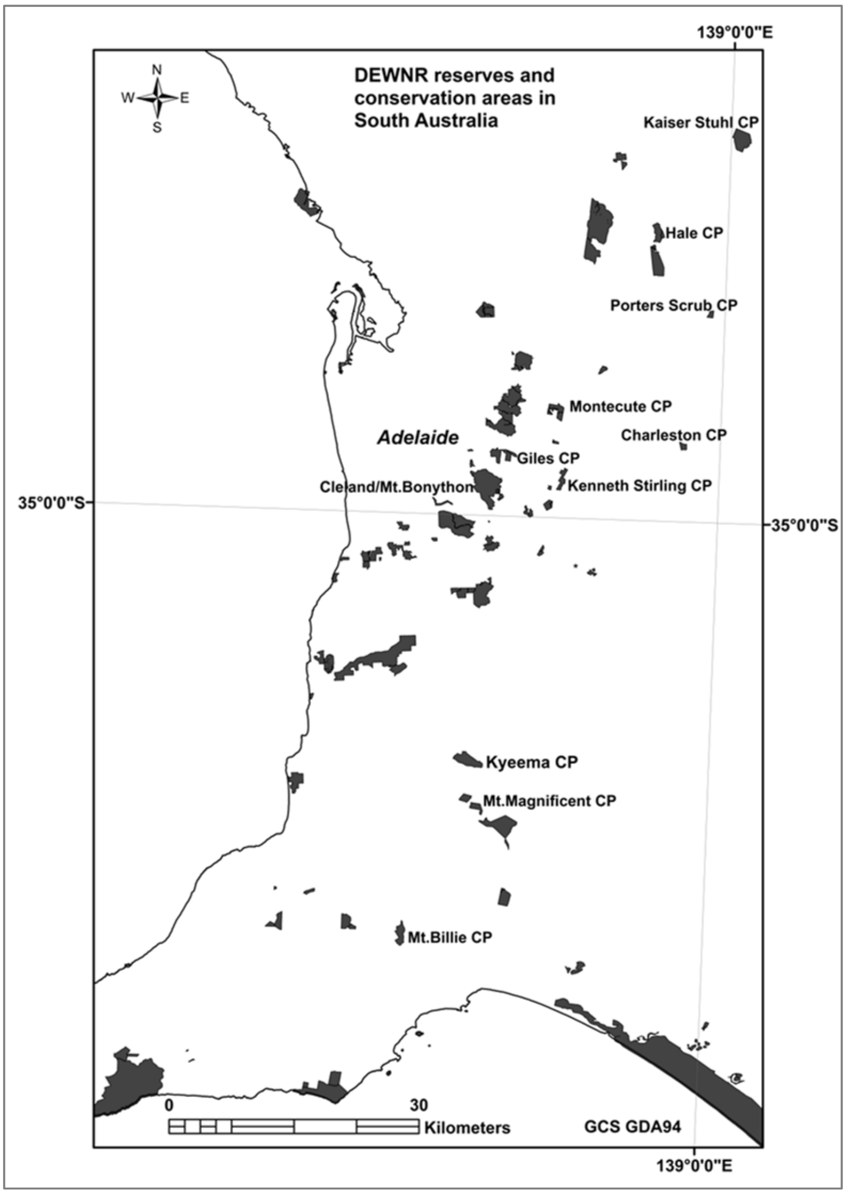

Figure 4.2 The locations of the thirteen field sites (labelled) within the South Australian park network. Two sites are located within both Giles CP and Cleland/Mt. Bonython. The map was constructed using ArcMap® sourced from Bureau of Meteorology (2007); Department of Environment Water and Natural Resources (2010c, 2010d)

Data analysis of actual presence and absence of blackberries in the MLR

Logistic regression mixed models were used to explore the relationships between the measured attributes of survey quadrats and the presence and absence of each blackberry taxon in native vegetation in order to understand the variation of ecological preferences among taxa. In these models, the sites were treated as a random grouping factor and the predictor variables were treated as fixed factors. These analyses account for the correlations among predictor variables. Analyses were conducted using the R statistical package ver. 3.1.1, The R stats package, R Core Team and contributors worldwide (2014), ggplot2 ver. 1.1.0, Wickham (2009), lme4' ver. 1.1.7, Bates *et al.* (2014), and dplyr ver. 0.2,Wickham and Francois (2014). We used Chi-squared statistics to examine the significance of deleted variables during model simplification in establishing the final simplified model to explain the occurrence of individual species of blackberry (Crawley, 2013). Boxplots were used to contrast the distribution of predictor variables in censused quadrats with and without the *R. fruticosus agg.* taxa.

Results

The most common species of blackberry found in Australia, *R. anglocandicans* (Evans & Weber, 2003), was also the most frequently encountered species in the survey of native vegetation in our study. In all, five of the ten species of blackberry that occur in the Mt. Lofty Ranges, were encountered in the sampling quadrats. *R. anglocandicans* was found in the greatest number ($n = 105$, 62.4%) of the 100m^2 quadrats that were found to have *R. fruticosus agg.* taxa present. *Rubus erythrops* Edees & A. Newton ($n = 42$, 24.8%) was the next most common species found in quadrats in native vegetation, followed by *Rubus rubritinctus* W.C.R.Watson ($n = 13$, 9.6%) and two species, *Rubus leucostachys* Schleich. ex Sm. and *Rubus laciniatus* Willd., were observed at relative percentages of 2.4% and 0.8% respectively (Fig. 4.3). Data show *R. laciniatus* and *R. leucostachys* occurred at a low frequency in survey quadrats across the majority of sites and *R. anglocandicans* was the most likely encountered invader of native vegetation communities (Fig. 4.3).

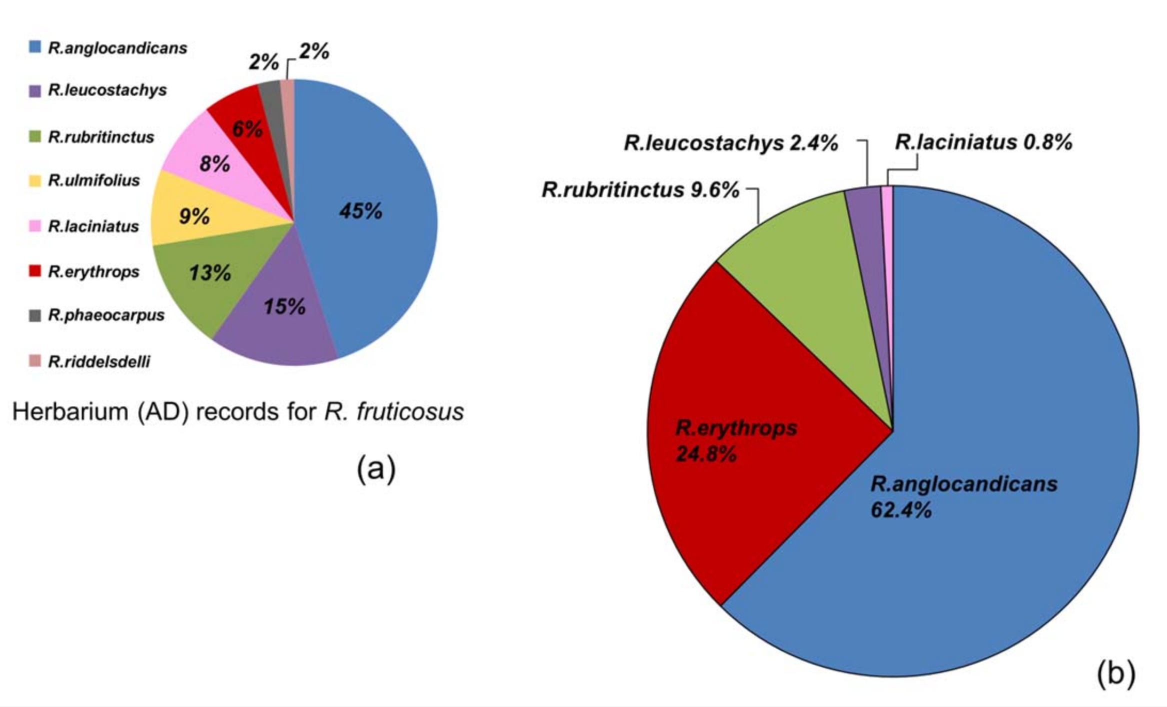

Figure 4.3 A comparison of *Rubus* taxa (a) from herbariam collections AD (by percentage of the total) and (b) the relative percentage of each *Rubus* species detected in quadrats from the field survey of native vegetation in the MLR

Modelling of presence/absence of blackberry species

We used the three species with the highest occurrences in the survey of 463 100m^2 quadrats for further analysis to explain the occurrence of individual *R. fruticosus agg.* taxa in the MLR.

Model outcomes for *R. anglocandicans*

The final logistic regression model to predict the presence of *R. anglocandicans* included five predictor variables (Table 4.2). Within the model, the positive effects of the maximum soil moisture percentage and the minimum soil moisture percentage suggest a reliance on mesic conditions in native vegetation communities. The variables maximum sunlight (LUM) and maximum bare ground percentage were negatively correlated with the occurrence of *R. anglocandicans,* suggesting that this species is less likely to be found in open sunny areas within native vegetation (Table 4.2, Fig 4.4). The latter terms were marginally non-significant on their own, but caused a significant drop in explanatory power when deleted from the model (Crawley, 2013). The aridity index, where high values of the index indicate more humid and wetter conditions, was positively correlated with the occurrence of *R. anglocandicans* but when deleted from the model, the decrease in model fit was marginally non-significant (*p=0.058*) and so the AI predictor was retained in the model.

Table 4.2 Summary of the generalised linear mixed model (GLMM) showing important variables correlated to the occurence of *R. anglocandicans* (n = 105) in native vegetation analysed against variation in site quadrats across the MLR

	Estimate	**Std. Error**	**z value**	**Pr(>|z|)**
(Intercept)	-0.433	1.335	-0.321	0.001
Aridity Index (AI)	3.147	1.868	1.685	0.092
Maximum bare ground %	-3.534	1.280	-2.762	0.006
Maximum soil moisture %	3.613	1.400	2.582	0.010
Maximum sunlight (LUM)	-1.647	1.013	-1.626	0.104
Minimum soil moisture %	3.491	1.337	2.612	0.009
Site (random factor)	Variance	Std. Dev.		
	2.715	1.648		

The boxplots of each predictor variable in quadrats defined by the presence and absence of *R. anglocandicans* in native vegetation reflect the outcomes of the model in predicting the occurrence of this species in the MLR. Overall, the presence of *R. anglocandicans* tends to be associated with wetter conditions (higher values of the Aridity Index indicate wetter conditions), higher levels of soil moisture, lower sunlight intensity and areas without extensive bare ground compared with sites where no blackberry occurred (Fig 4.4).

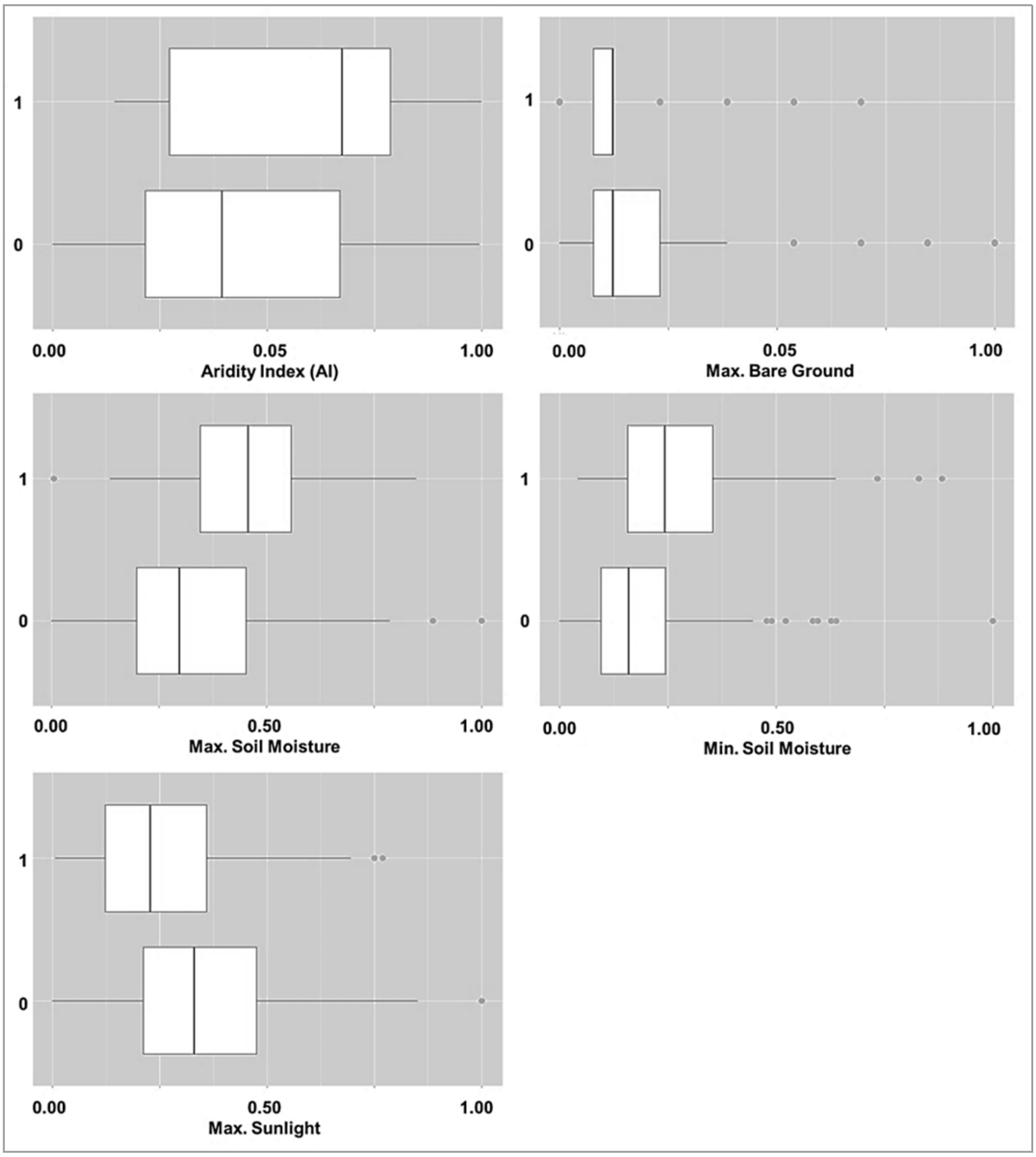

Figure 4.4 Summary boxplots of significant variables which contribute most to the model output of *R. anglocandicans.* Y axis: 0=absence 1=presence of *R. anglocandicans*

Model outcomes for *R. erythrops*

The occurrence of *R. erythrops* can be predicted by three habitat variables (Table 4.3). Aridity (AI) where higher values represent more humid and wetter conditions, was the most significant contributing variable to explain the occurrence of this species (Table 4.3, Fig. 4.5). The presence of *R. erythrops* in the quadrats was significantly negatively correlated with percentage of maximum sunlight and was positively correlated with minimum soil moisture availability, which in the field relates to the level of soil moisture in summer in native vegetation communities.

Table 4.3 Summary of the generalised linear mixed model (GLMM) showing important variables correlated to the occurence of *R. erythrops* (n = 42) in native vegetation

	Estimate	**Std. Error**	**z value**	**Pr(>|z|)**
(Intercept)	-5.264	1.194	-4.409	1.04e-05
Aridity Index (AI)	4.679	1.506	3.106	0.002
Maximum sunlight (LUM)	-2.718	1.417	-1.919	0.055
Minimum soil moisture %	3.942	1.529	2.579	0.001
Site (random factor)	Variance	Std. Dev.		
	0.9777	0.9888		

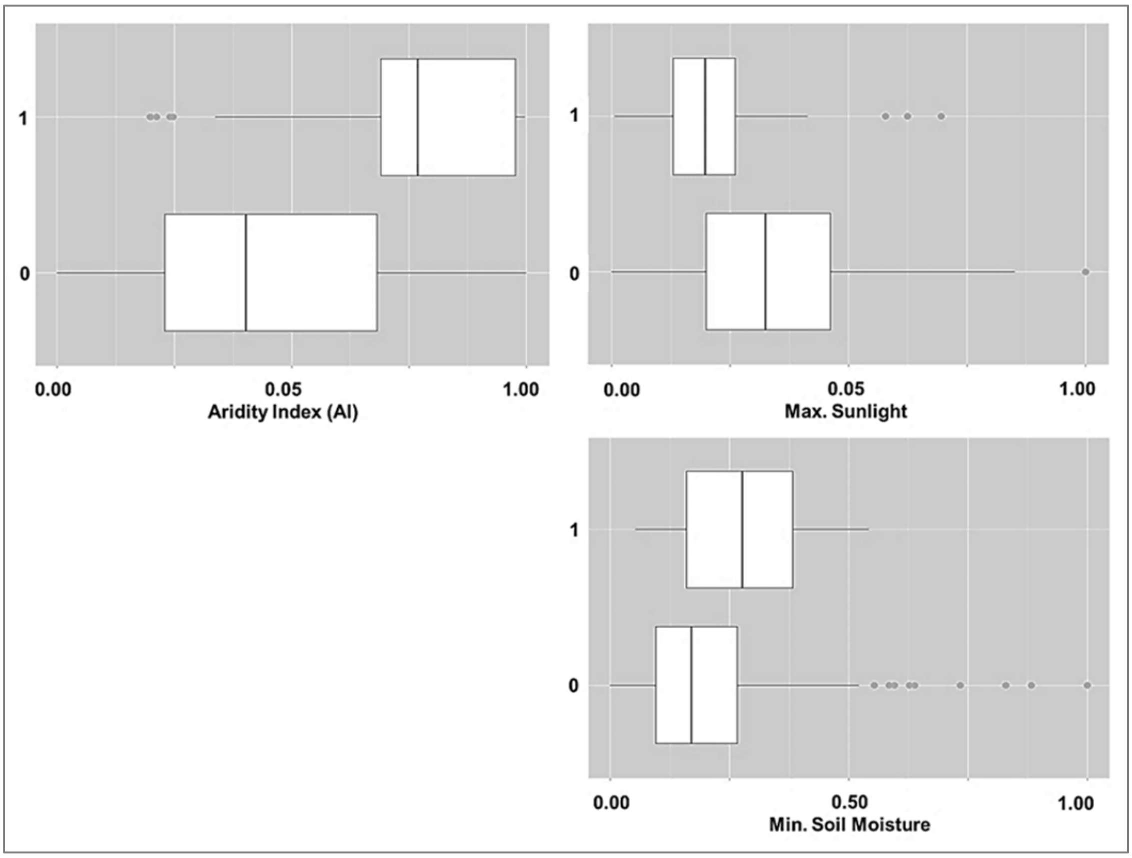

Figure 4.5 Summary boxplots of the significant variables which contribute most to the model output of *R. erythrops.* Y axis: 0=absence 1=presence of *R. erythrops*

Model outcomes for *R. rubritinctus*

The model for *R. rubritinctus* shows the presence of this species in the survey is negatively correlated with topographic slope and percentage of bare ground (Table 4.4). The summary boxplots (Fig 4.6) show *R. rubritinctus* is more likely to occur in quadrats that are more flat in topography, that have relatively moist soils and that are relatively less sunny. The effect of the presence of broom, although a significant predictor, is not obvious from the boxplot and the sample size for this blackberry species is quite low.

Table 4.4 Summary of the generalised linear mixed model (GLMM) showing important variables correlated to the occurrence of *R. rubritinctus* (n =13) in native vegetation

	Estimate	**Std. Error**	**z value**	**Pr(>|z|)**
(Intercept)	-8.481	5.253	-1.614	0.106
Slope (degrees)	-4.845	1.678	-2.887	0.004
Broom density %	2.305	1.087	2.120	0.034
Maximum bare ground %	-10.138	6.218	-1.631	0.103
Minimum soil moisture %	8.991	3.879	2.318	0.020
Site (random factor)	Variance	Std. Dev.		
	40.24	6.343		

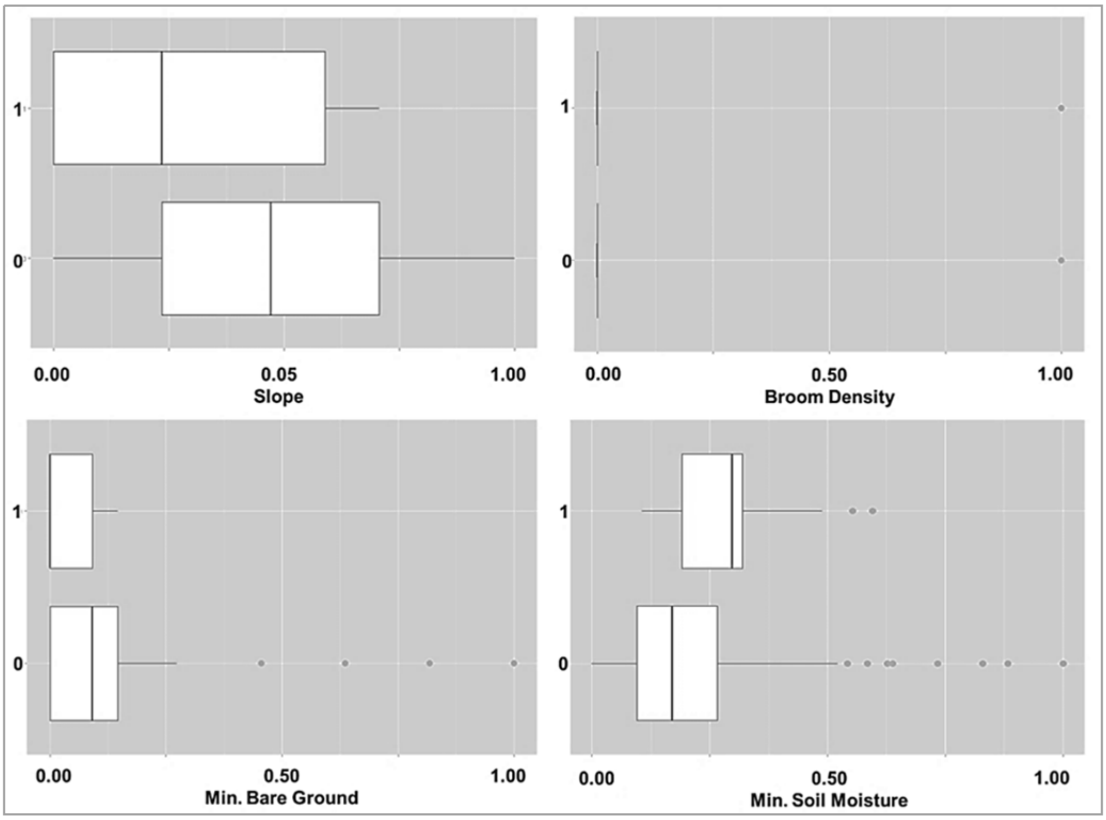

Figure 4.6 Summary boxplots of the important variables which contribute most to the model output of *R. rubritinctus*. Y axis: 0=absence 1=presence of *R. rubritinctus*

Discussion

Results from the analyses of our survey demonstrate blackberry species invade native vegetation communities differently and their distribution throughout the Mt. Lofty Ranges is influenced by landscape topography, climate tolerances and discrete biotic attributes of each species.

Rubus anglocandicans, R. erythrops and *R. rubritinctus* appear to be the most common invaders of native vegetation communities of the ten species found in the Mt. Lofty Ranges. Several factors significantly influenced the occurrence of individual species of blackberry enabling us to describe habitat preferences for each of the identified species invading vegetation communities.

Knowledge of the soil constraints of the Australian taxa is lacking except for those recorded by Amor (1973), who found that species grew in loamy acid soil of low fertility but more generally *R. fruticosus* agg. species were found in a variety of soils of differing textures and pH in Victoria (Amor 1971). While we did not test either the soil type or pH of soil in the Mt. Lofty Ranges where blackberries occurred, the limited knowledge of the preference of *Rubus vestitus* Weihe (not occurring in S.A.) for calciferous soils (Taylor, 2005), for example, appears to be specific for this species in its ecological context in Great Britain and Europe and not representative of all members of the *R. fruticosus* agg.

The historic information (Watson, 1958; Amor, 1974; Taylor, 2005) supports the frequency of occurrence along streamlines of blackberry taxa maintaining annual minimum soil moisture.

Different relative frequencies of occurrence of *Rubus* species occurring in the Mt Lofty Ranges were observed when herbarium (AD) datasets were compared to our survey results. This was evident by the virtual absence of *R. leucostachys* in the field survey of native plant communities whereas *R. leucostachys* was the second most commonly found species in herbarium (AD) datasets. This implies that *R. leucostachys* is more common in open and disturbed areas than in areas of native vegetation.

Rubus anglocandicans *habitat preferences*

Analysis of the significant factors, which predict the presence of *R. anglocandicans* in native vegetation, suggest wetter native vegetation types (such as sclerophyll forest) are more likely to be invaded by *R. anglocandicans* where summer and winter soil-moisture conditions remain favourable. This factor may be important in affecting the ability of this species to propagate and increase its population rapidly through vegetative spread (Watson, 1958). The negative correlation of sunlight intensity with the presence of *Rubus anglocandicans* implies this species prefers more shaded habitats in native vegetation, suggesting that *R. anglocandicans* is prone to be limited by the intensity of sunlight at the margins of more dense vegetation and by gaps in forest canopies. In examples in the literature, Amor (1973) suggested that the penetration of light into vegetation was a factor reducing the extent of *R. procerus* infiltration into forestry plots in Victoria, Australia but he went on to say *R. procerus* had less critical requirements for water nutrients and light than *R. laciniatus*. A study by Lindenmayer and McCarthy (2001) though found survey plots (7% of 507) located in continuous native vegetation in Victoria, Australia were significantly at risk of invasion by *R. fruticosus* agg. when compared with more open area plots in adjacent forest, and this provides supportive evidence for our findings.

The positive correlation of soil moisture during winter and particularly during summer months with the presence of *R. anglocandicans* implies that this taxon occupies and persists in mesic areas, which may advantage the spread by vegetative reproduction. Amor (1974) described the process of the development of daughter plants from tip rooting primocanes in a single species *R. procerus* in Australia, however, he did not investigate the processes and mechanisms as to how the invasive capacity of the *R. fruticosus agg.* might be enhanced through these mechanisms in invading native vegetation. For almost all species of the *R. fruticosus* agg., vegetative spread appears to rely upon sufficient soil moisture levels (noted by Edees & Newton, 1988) and may be the dominant mechanism of dispersal within native vegetation communities once the species have become established but further testing of this question is needed, particularly in the Australian context.

Rubus erythrops *invasion into native vegetation*

The second most common blackberry species detected by our survey of native vegetation sites was *R. erythrops*. The occurrence of *R. erythrops* in native vegetation appears to be associated with wetter conditions that prevail in the MLR. *Rubus erythrops* also prefers more shaded areas within native vegetation and is not tolerant of habitats with intense summer sunlight. While little information has been reported as to the habitat preferences of this species and of other exotic blackberries within Australia and elsewhere, Edees and Newton (1988) observed this species to be distributed in woodland and heath areas in Britain. Results of the field survey suggest that *R. erythrops* is adaptable in a complex topographical area such as the Mt. Lofty Ranges, where many of important parks and reserves of high diversity value are located, and is able to invade areas of native vegetation.

Rubus rubritinctus *invasion into native vegetation*

The statistical modelling predicting the occurrence of *R. rubritinctus* in native vegetation appears less robust than the models for the other *Rubus* taxa and we note that the interpretation is based upon a small dataset for this species and therefore needs to be treated with caution. Nevertheless, statistical analysis of this species suggests this species' occurrence correlates with habitats of low gradient and like the other closely related exotic *R. fruticosus* agg. taxa above, its presence is also associated with greater minimum soil moisture in native vegetation areas. Herbarium collections of this species (AD) have been commonly sourced in areas and from populations occupying the wide flat valleys that are a feature of the eastern side of the Mt. Lofty Ranges. Further study of this species though is needed to expand the understanding of the habitat preferences.

Broader considerations of blackberry invasion success

While the ecological attributes of exotic blackberries are largely unstudied in their introduced ranges, the study of more general processes influencing invasion success of exotic taxa, such as margin or edge effects, have long been viewed to enhance the invasion of weed species (Honnay *et al.*, 2002; Harper *et al.*, 2005). These margin effects appear to be a major factor in the success of some invasive plants in their introduced ranges but results are mixed in studies in undisturbed habitats (Vilà & Ibáñez, 2011; Saure *et al.*, 2013). With regard to blackberry taxa in Australia, Lindenmayer and McCarthy (2001) noted in their study of invaded pine-eucalyptus mosaics, that highly disturbed *Eucalyptus* remnant communities located close to edges of pine plantations were the most likely to suffer *R. fruticosus* agg. invasion, suggesting edge effects do play some part in the establishment of *R. fruticosus* agg. taxa within native vegetation. In contrast, they did not suggest an ecological mechanism to explain the invasion of plants of the *R. fruticosus* agg. that were found in plots located deep in native vegetation. Our study sites within the Mt Lofty Ranges are large continuous sections of native vegetation with relatively small margins.

Complex interactions and performance in margins and gaps in native vegetation habitats by individual blackberry species has not been fully explored for the Australian taxa. Amor (1973, 1974) investigated the vegetative spread of *R. fruticosus* agg. in open areas around Frankston in Victoria and suggested more work was required as to the light, soil and moisture preferences of *R. fruticosus* agg. taxa in order to form a clearer understanding of the pathways of introduction and spread into native habitats in Australia.

Cadenasso and Pickett (2000) in a study of native taxa in the United States, provided some evidence to suggest that the flux of seed dispersal into the forest interior from margins and gaps was more correlated to the shape and structure of margin areas, which may be helpful in connecting the processes of establishment with spread in weed invasions particularly for *R. fruticosus* agg. taxa that remains unresolved.

Results of our survey suggest that invasion processes into undisturbed vegetation are strongly influenced by the biological preferences of each species and therefore are partially trait driven. For example, the relatively wide range of ecological tolerance to aridity of *R. anglocandicans* within habitats in the MLR is consistent with the observation that this species is able to persist in a greater range of habitats over larger areas in the Australian landscape (Evans & Weber, 2003). *Rubus anglocandicans* appears not to be tolerant of open areas, which may have particularly low soil moisture under summer conditions, and which may limit its expansion into open unwooded areas. The specific preference of *R. anglocandicans* to avoid high sunlight intensities, e.g. presumably in forest gaps, poses some interesting questions about the processes that affect population expansion once species become established.

Comparing niche characteristics of R. anglocandicans, R. erythrops *and* R. rubritinctus

Overall, modelling of the three blackberry species from the presence/absence field survey of native vegetation clearly indicates the different habitat characters that influence their occurrence in their introduced ranges. While the populations of all three blackberry species occur in the Mt. Lofty Ranges and their distributions overlap, results presented here support the assumption that *R. anglocandicans* is more capable of expansion into native vegetation under current conditions and climatic regimes in this area, while the second most common species in native vegetation, *R. erythrops*, tends to occur in wetter areas. *Rubus rubritinctus* is a less common species in native vegetation but appears to be more likely to occupy wider valleys in the Eastern areas of the Mt. Lofty Ranges.

By comparison, the study of the fundamental niche in Chapter 2 showed the potential range of *R. anglocandicans* was the most extensive with the exception of *R. leucostachys*. *R. anglocandicans* was also the most different in the niche identity analysis from other species in Chapter 3.

The differentiation of the species niches in the results in Chapter 3 suggests the niches for *R. anglocandicans* and *R. leucostachys* are not identical, while the results presented here from the field survey of native vegetation show that *R. leucostachys* does not often occur in native vegetation. *R. erythrops* on the other hand appears to occupy sites characterised by a narrower range of aridity index values than *R. anglocandicans* based on the field survey of native vegetation.

Acknowledgements

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Appendix (App.) Chapter 4

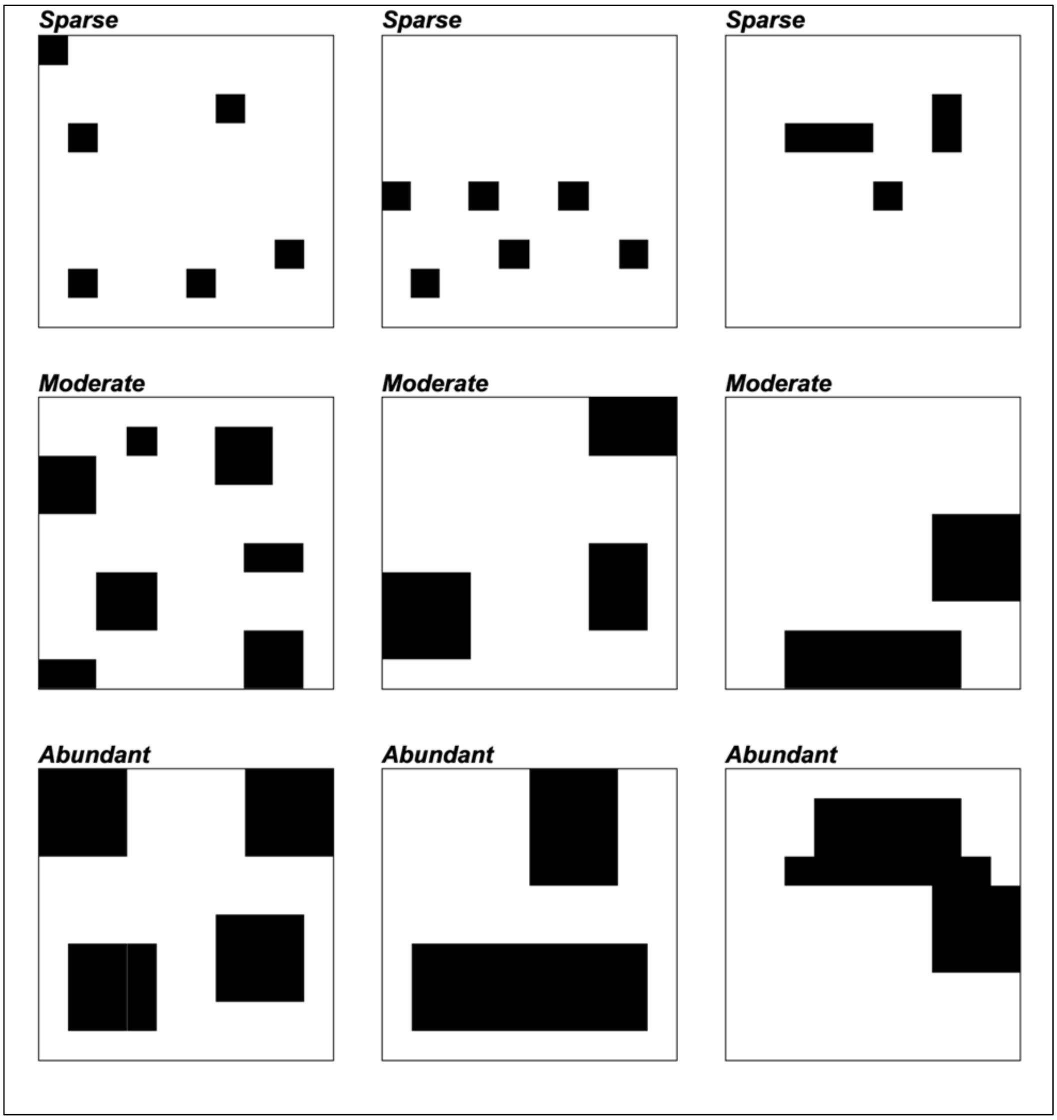

Figure A4.1 Binary matrix table used for field assessment of relative abundance of blackberry in quadrats

Visual assessment of blackberry is a simple way of rating weed abundance. To improve the assessment in the field compare the different base patterns from left to right was used for each density estimate in sample quadrats. The binary matrix was modified from methods by McNaught *et al.* (2008).

Definitions-Figure A4.1

The definitions of the density patterns were constructed by assessing the growth stages and based on knowledge of the biology of blackberry.

Sparse

Single primocanes of first year growth are, usually found for example at the base of trees at road sides, in open fields or along creek lines, and as a result of bird drop, also found in native vegetation. Plants are often represented by a single primocane and do not form clumps and represent the first stage of invasion through seed dispersal by animals or the action of surface flows of water from climatic events (indicates the extent of larger invasions). Density is very low down to one plant in $100m^2$. All parts of plants are easily accessible.

Moderate

Plants have formed into regular distinct clumps greater than 1 metre in diameter. Clumps are irregular in shape. Clumps may be the result of overlapping growth of more than one plant. Individual primocanes are not difficult to distinguish. Clumps are separated by open ground or native vegetation with usually greater than two plants in $20m^2$. Infestations along creek lines are not continuous. Access to individual clumps is more difficult with peripheral parts reachable but requires entry into the clump.

Abundant

Plants have formed into clumps or continuous carpets greater than 4 metres in diameter with some irregular in shape; this may be the result of overlapping growth of more than one plant. Individual primocanes are often very difficult to distinguish. Clumps are often not separated by open ground or native vegetation and individual plants are often not distinguishable. Infestations along creek lines and in native vegetation are continuous. Access to the plants is only possible at the periphery of the infestation.

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Chapter 5

Competitive effects of invasive blackberries on selected native Australian plant species.

[1]John Marshall, [2] Molly A. Whalen, and [3] Duncan A. Mackay

[1, 2, 3] Flinders University, School of Biological Sciences, GPO Box 2100, Adelaide, S.A. 5001, Australia.

Abstract

Aim We test the hypothesis that exotic blackberry taxa will out-compete Australian native plant taxa

Location Mt. Lofty Ranges, South Australia

Methods We performed paired competition experiments between exotic blackberry species (*Rubus anglocandicans*; *Rubus erythrops* and *Rubus leucostachys*) and two Australian natives (*Leptospernum continentale* and *Acacia provincialis*) in shade house experiments and in the field.

Results The growth of *L. continentale* paired with one or more of the three blackberry treatments under shade house conditions showed highly significant effects on growth measured as above-ground and below-ground weights of plants (both wet and dry weights with the exception of root dry weight), height increase, and increase in stem area after a four-month growth period compared to *L. continentale* growing on its own. The growth of *A. provincialis* (which is considered to be a biological nitrogen fixer) under shade house conditions showed no significant effects in terms of reduced plant vigour when grown with three blackberry species measured over the same period. The pattern of effects on the vigour of plants of *L. continentale* grown with three blackberry species was consistent over all six metrics measured, showing *R. anglocandicans* had the strongest effect and *R. leucostachys* the weakest effect on *L continentale*. Analysis of growth responses from the field experiments of the two native species growing with blackberry species compared to control planting did not provide a clear understanding of the competitive responses of the two native species (*A. provincialis* and *L. continentale*) in the field due to confounding effects of herbivory.

Main Conclusions The impact on the growth performance of the two native species in the shade house experiment, when they were combined with three blackberry species (*R. anglocandicans*, *R. erythrops* and *R. leucostachys*), suggests plant-plant interactions and the biological traits of individual taxa may be important in providing some protection to invasion by blackberry species.

Keywords Invasion, plant communities, competition, blackberries, Australia, *Rubus fruticosus*, nitrogen fixation

Introduction

The invasion of native plant communities by alien plants and the subsequent changes to ecosystem structure, function and community composition is an ever-increasing threat to the maintenance of biodiversity (MacDougall *et al.*, 2009; Powell *et al.*, 2011; Marrs *et al.*, 2013).

Generally, invasive species represent threats to biodiversity and ecosystems but they also provide an opportunity to understand more about complex processes such as competition that limit or facilitate the spread of invasive species (Crooks, 2002).

Exotic taxa in introduced ranges are expected to be disadvantaged by competition in highly bio-diverse communities but the empirical evidence that supports this hypothesis is mixed and suggests a highly complex group of processes and plant interactions determines whether or not an alien taxon becomes successful in new areas (Maestre *et al.*, 2006; MacDougall *et al.*, 2009; Jeschke *et al.*, 2012; Parker *et al.*, 2013). Competition within productive communities is affected not only by the ability of plant taxa to acquire resources but also by plant-plant interactions and the inherent morphological plasticity of some invasive taxa (MacDougall *et al.*, 2009; Navas & Violle, 2009; Gallagher *et al.*, 2010; Parker *et al.*, 2013). For example, the morphological plasticity displayed by some Australian weeds, such as *Chrysanthemoides monilifera* subsp. *rotundata* and *Cytisus scoparius* Link (Scotch Broom), suggests weed species exhibit both multiple life history strategies and a variety of adaptations in their introduced range (Downey & Smith, 2000; Gosper, 2004). Superior competition for light for example, appears from these studies to influence the population expansion of *C. scoparius* and *C. monilifera* but it is likely that response to light competition is only one of a group of linked functional traits that enables these species to become superior competitors. *Cytisus scoparius* for example, is an exotic leguminous shrub native to Europe (Crooks, 2002), and like many other legumes is likely to be capable of biological nitrogen fixation (BNF) (Crews, 1999). In plant communities, nitrogen fixation is suggested to impart superior competitiveness to some invasive taxa (Yelenik *et al.*, 2004). Other introduced taxa have displayed similar adaptability to biomes within Australia where Gallagher *et al.* (2010) studied 26 exotic taxa and found the majority (19) extended their range of climatic tolerances in new habitats compared with their native ranges. Gallagher *et al.* (2010) though did not expand on the underlying mechanisms that enable these taxa to invade new areas. Considering the extended capacity of exotic taxa in this study to invade and extend their ranges in Australia, even highly diverse communities appear less competitive and particularly at risk of invasion from introduced plants.

Understanding the competitive abilities of invasive plants in new habitats is linked to the complex physiology and traits of invaders. More recent studies in weed research (Gioria & Osborne, 2014) suggest invasive species are multi-dimensional in gaining dominance over native species but there are considerable knowledge gaps in the understanding of the complexities of competition both at the individual level and community level, and are likely to involve species specific interactions between natives and invaders (Richardson, 2011; Prins & Gordon, 2014).

Biology and ecology of invasive blackberries

European blackberries (*Rubus fruticosus* L. aggregate), are invasive weeds globally but very little is known about the underlying biology and ecology of individual species within the aggregate that aids them in becoming successful invaders. Invasive taxa of the *R. fruticosus* agg. display a diversity of invasion globally and are successful invaders, where different species of the aggregate have invaded a variety of habitats and appear to be adapting to biomes different to that of their native ranges (Groves, 1998). The diversity of invasive taxa within the *R. fruticosus* agg. suggests blackberries have a suite of competitor strategies and morphological traits that advantage them in introduced ranges.

Almost all taxa in the *R. fruticosus* agg. are perennial woodland species in their native ranges (British Isles, Europe, North America and Asian regions) occurring mainly at the margins of woodland vegetation. Blackberries are adaptable to a wide range of habitats and in particular, are colonisers of disturbed land in both their native and introduced ranges (Edees & Newton, 1988; Clark *et al.*, 2007).

Very little is known about the interactions of *Rubus* taxa with soil, both in their native and introduced ranges. The soil preferences of a very limited number of species have been examined (Taylor, 2005; Caplan & Yeakley, 2006; Renteria *et al.*, 2012). Several early studies investigated the nitrogen fixing abilities of *Rubus ellipticus* Sm. (*Rubus* subgenus: *Idaeobatus*) and suggested *R. ellipticus* formed symbiotic relationships with soil microbes but this was subsequently rejected in other studies where plants were found to be devoid of root nodules and unable to capture atmospheric nitrogen (Becking, 1979; Chaudhary *et al.*, 1985; Chaudhary & Mirza, 1987).

Most members of the *R. fruticosus* agg. have a complex breeding system reproducing by vegetative spread and through a process called apomixis (Koltunow & Grossniklaus, 2003). Apomixis is a complex asexual mode of reproduction that generates large numbers of highly fecund seed on the maternal line without gamete reduction and is often associated with polyploidy (duplication of the genome) (Nogler, 1984). The more common vegetative spread amongst species occurs through underground sucker development, vegetative layering, and tip rooting of the primary canes of established plants in the autumn (Weber, 1999; Evans *et al.*, 2007).

Blackberries in Australia

In Australia about 15 species of the *R. fruticosus agg.* have been introduced where species such as *Rubus anglocandicans* A. Newton, identified as the most widespread species, appears strongly clonal (with both vegetative spread and production of clonal seed) (Evans & Weber, 2003; Evans *et al.*, 2007). Very little work has been undertaken to examine the ecology and biology of different blackberry species and specifically as to why some taxa appear to be superior competitors within native plant communities (Evans & Weber, 2003; Caplan & Yeakley, 2013; French *et al.*, 2014). Amor and colleagues (Amor, 1973, 1974; Amor & Miles, 1974; Amor *et al.*, 1998) original work forms the basis of the knowledge of the ecology of the *R. fruticosus agg.* in Australia, with a perspective on improving management in forestry plantations where blackberries had invaded but is limited in its contemporary value as only a very small number of species were investigated using names applied prior to a more recent taxonomic study of the aggregate in Australia (Evans *et al.*, 2007).

Control and management within Australia

The introduced species of blackberry within Australia are a major weed problem, and they degrade biodiversity of natural systems and impact adversely on agriculture. Blackberries along with other weed taxa in Australia are responsible for major losses to primary production, estimated to cost more than $4 billion annually (Davies, 1998; Agriculture & Resource Management Council of Australia & New Zealand & Australian & New Zealand Environment & Conservation Council and Forestry Ministers, 2000; Sinden *et al.*, 2004). Known as one of the Weeds of National Significance (WONS), management systems have focussed strongly on the aggregate as a whole due mainly to lack of ecological and biological knowledge pertaining to individual species and the primary need to find solutions to the agricultural threat they pose (Evans & Weber, 2003; French *et al.*, 2014).

Blackberry weed research

A few international studies of closely related blackberry species have attempted to understand the underlying traits linked to invasion success and to show how introduced competitors may out-compete related native species on multiple functional levels. Two studies of the invasive species *Rubus armeniacus* Focke (syn. *Rubus discolour* Weihe & Nees) in the Pacific north-west of the United States, demonstrated the superior competitive performance of *R. armeniacus* over the native *Rubus ursinus* Cham. and Schlect. in native vegetation (Yeakley & Caplan, 2008; Caplan & Yeakley, 2010, 2013). *Rubus armeniacus* displayed lower reproductive effort and less water stress under drought conditions than the native species (*R. ursinus*). Tolerance to dry conditions and a superior photosynthetic capacity compared to *R. ursinus* showed the invasive species was a functionally superior competitor (Caplan & Yeakley, 2013). Superior functional morphology in leaf-level photosynthetic capacity also appeared to advantage *R. armeniacus* in a study in the same region, as did resource-use efficiency (McDowell, 2002).

The role played by inherent functional morphological traits in contributing to successful invasion by non-native species remains unclear, e.g. as to whether there exists a single set of traits for all invasive species (Drenovsky *et al.*, 2012). Pathways of introduction and the origins of invasive species also appear to be important for naturalisation success in new ranges (Lambdon & Hulme, 2006; Lloret *et al.*, 2004; Lloret *et al.*, 2005).

Aim

We aim in our study to test the competitive responses of three invasive species of the *R. fruticosus* agg. *Rubus anglocandicans* A. Newton, *Rubus erythrops* Edees & A. Newton and *Rubus leucostachys* Schleich. Ex Sm. on the growth of two native species *Leptospernum continentale* and *Acacia provincialis*.

We hypothesise that the exotic blackberry taxa would out-compete the native plant taxa both in controlled conditions and in invaded native vegetation communities within the Mt. Lofty Ranges, South Australia and further, that the most widespread invasive species of *Rubus* in Australia *R. anglocandicans* (Evans & Weber, 2003) will compete more strongly against the two native taxa than either *R. erythrops* or *R. leucostachys.*

Material and Methods

Considerations in greenhouse studies of plant competition

Greenhouse studies of plant competition are preliminary experiments undertaken to try to understand the complex interactions that exist between plants in the field and make some competitive and others not.

The method of measurement of plant competition of individual plants in mixtures in shadehouse/greenhouse studies has been the subject of intense debate as to the best design for interpretation of experimental results (Mead, 1979; Freckleton & Watkinson, 1999; Gibson *et al.*, 1999; Cousens, 2000; Freckleton & Watkinson, 2000). Cousens (2000) promotes the idea that pot experiments are an attempt to understand what might happen in the field but there are many variations in experimental designs (Cousens *et al.,* 1991, 2003). Additive designs for pot experiments in greenhouse studies appear to offer the simplest designs but results may be limited in their interpretation (Vilà & Weiner, 2004). A simple asymmetric pair-wise design (also called an additive or equal proportion; Austin *et al.* (1988)) was chosen here to measure the relative yield of both natives over a short four month period while blackberries are in their rapid growth phase in spring and summer. An alternative experimental design approach using reciprocal or replacement series designs (Gibson *et al.*, 1999; Vanclay, 2006) does not allow an unambiguous interpretation of the influence on individual biomass of intra-specific or inter-specific competition between the native and introduced species (Underwood, 1997, pg. 414; Connolly *et al.*, 2001).

Native competitor species

Two species of natives (*Acacia provincialis* A. Camus and *Leptospermum continentale* Joy Thomps.), were purchased from a commercial supplier (ERA Nurseries, Hamilton. Victoria) as tube stock. Plants of *Acacia provincialis* had root nodules attached indicating nitrogen fixing capability. Plants of each species were grown from seed and were approximately 18 months of age at the start of the experiments. *Acacia provincialis* is a fast growing tree, and a variable species influenced by environmental conditions. Habit is slender to more densely spreading, with plants growing to 10m tall in fertile wet soils (O'Leary, 2007). *Leptospermum continentale* is a shrub up to 2m tall, sometimes taller, influenced by habitat conditions. Plants have an erect, densely branching habit and grow in most soil types and in poorly drained areas (Thompson, 1989). Both of these species commonly occur with species of *R. fruticosus* agg. in the Mt. Lofty Ranges.

We chose these species, as they are indigenous to the local area in the Mt. Lofty Ranges and are fast growing natives tolerant of a wide range of conditions. Both species are common taxa in native plant communities invaded by blackberries within the Mt. Lofty Ranges. In addition, *A. provincialis* was included as a nitrogen fixer (Turnbull, 1986) and this trait has been suggested to enhance competitive ability in plants (Brooks, 2003; Castro-Díez *et al.*, 2014). Both of the native species are widely distributed throughout southern Australia and are known for their weedy characteristics in other ranges particularly *A. provincialis,* which has become a serious weed for example in the Iberian Peninsula, on some Mediterranean island communities and in the South Africa Fynbos (Webber *et al.*, 2011; Correia *et al.*, 2014). Aspects of the reproductive biology and ecology of some species of Australian acacias are well-established (Kenrick & Knox, 1985; Sedgley, 1986; Turnbull, 1986). Australian *Acacia* spp. may act as mediators to invasion within invaded plant communities but the mechanisms that influence this mediation are still unclear (Gibson *et al.*, 2011).

Blackberries used for field and shade house experiments

Of the ca. fifteen blackberry species found in Australia, ten occur in South Australia (Evans *et al.*, 2007). Of these ten species only five species were observed invading native vegetation in our field survey within the Mt. Lofty Ranges (Chapter 4).

Members of the *R. fruticosus* agg. are typically shrubs with non-flowering stems and canes being produced in the first year. In the second year of growth, flowering stems are produced. Habit is variable between species and stems and canes may be high arching or ground spreading. Vegetative spread of most species occurs in the autumn when cane apices touching the ground tips root and divide forming new plants radiating around the original plant (Amor, 1974; Evans *et al.*, 2007).

Three species were selected from this group of five for our competition study, these being *R. anglocandicans, R. erythrops,* and *R. leucostachys.*

The most frequently encountered species in native vegetation surveyed in our field survey, *R. anglocandicans,* which was also the most commonly represented species in herbarium records (AD)), was included, as was *R. erythrops,* the second most common invader of native vegetation in our field survey and a species that was often found in deeply shaded areas under forest canopy. The third species studied, *R. leucostachys*, which represented the second most common species in herbarium records (AD), was rarely encountered in our field survey of native vegetation.

In the field experiment we used only *R. anglocandicans* and *R. erythrops* at two invaded sites in the Mt. Lofty Ranges for companion plantings with the two native species. These experiments were performed over a four and a half month period from September 2013 until January 2014.

Shade-house competition experiment

The effects of blackberry species on native plant species were examined by planting native plant species alone and in combination with the three blackberry species.

We measured the growth of the native species under a simulated forest canopy cover in a shade house environment (controlled shade house conditions under 50% shade-cloth). Plants of the native species *L. continentale* and *A. provincialis* were co-potted with *R. anglocandicans*, *R. erythrops, and R. leucostachys* tip-root stock.

Blackberry root stock

We collected five to ten ramets of the different species at separate locations within the Mt. Lofty Ranges in the winter (Fig 5.2). In all, we gathered more than fifteen genets of each blackberry species used in the shade house experiment. We collected for each blackberry taxa thirty to thirty-five live tip-roots from primocanes in the winter of 2013. Each collection was identified using Barker and Barker (2005). Voucher specimens for genets were deposited at the South Australian State Herbarium (AD). Tip-roots were washed free of soil from the field and each ramet was then potted in ten centimeter pots using coco-peat potting mix (Waite Agricultural Research Institute). We grew the blackberry rootstock for approximately two months in a shade house environment to ensure new plant vigour.

Potting methods

Before co-potting occurred, we recorded the weight, height (from the soil surface) and the diameter of the stem 1 cm above the soil surface, of the blackberry rootstock and the two native species. Blackberry tip-roots were weighed without soil attached while the native species were weighed after removal from the tube without disturbing the root ball.

For each of the two native plant species, twenty individuals were potted on their own as controls, and thirty individuals were potted with each of the three blackberry species, giving a total of 110 pots of each native plant species. Plants were potted in twenty-centimeter diameter plastic pots with a standard volume of coco-peat potting soil mix containing slow release fertiliser that was suitable for native Australian plants (Fig 5.1).

We grew potted plants within a shade house covered in 50% shade cloth and with a watering cycle of three minutes every six hours. Pots were rotated within the shade house fortnightly to limit local shading and watering effects.

Figure 5.1 Image of co-potted blackberries with the two native species, *A. provincialis,* (top left) and *L. continentale* (top right) and controls, (bottom left and bottom right), within the experimental shade-house environment

The shade house plants were harvested after four months and the above-ground height and the stem diameter (one cm above the soil surface) of *A. provincialis* and *L. continentale* plants were measured. We separated the roots and above-ground material (leaves and stems) of both the native and blackberry plants and recorded the wet and dry weights of each plant.

Field competition experiment

We carried out a competition experiment in the Mt. Lofty Ranges, South Australia to test the effects on growth of *A. provincialis* and *L. continentale* planted with two blackberry species within native plant communities invaded by blackberries (Fig 5.2).

A diversity of vegetation communities exists within the Mt. Lofty Ranges region including sclerophyll forests, drier heathy woodlands, grassy woodlands, grasslands, mallee, swamps, wetlands and various coastal and estuarine ecosystems (Department for Environment and Heritage, 2009). We chose two sites of sclerophyll forest between 600 and 680 metres in altitude, at Mt. Bonython and the Greenhill Road Reserve (lat.-34.957906 lon.138.713933; lat.-34.957906 lon.138.957906). These sites are typical of the vegetation structure and vegetation types found at upper elevations in the Mt. Lofty Ranges (Fig. 5.3).

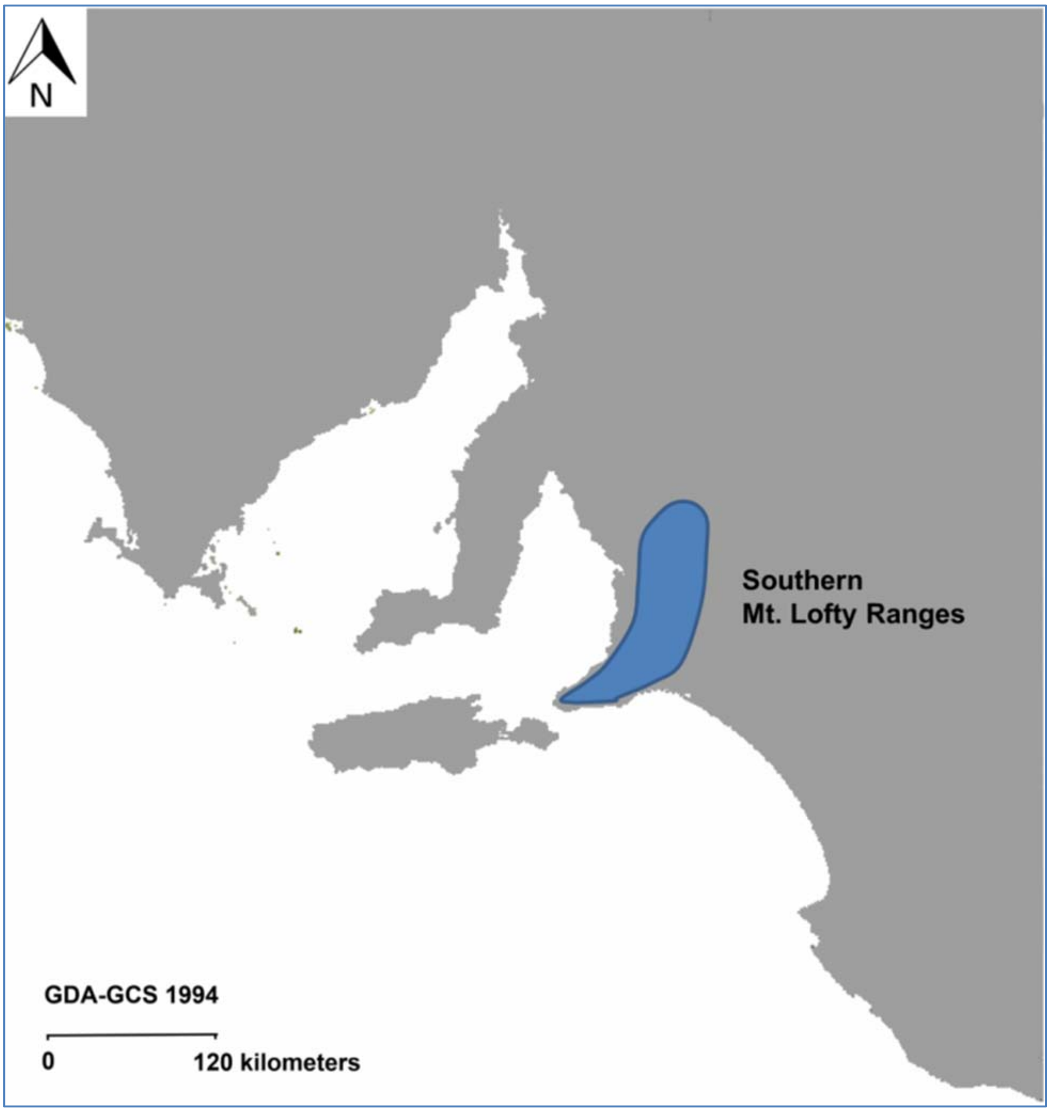


Figure 5.2 Location map of the Mt. Lofty Ranges, elements sourced from Geoscience Australia (2006)

Both sites have a mature over-story of *Eucalyptus baxteri* (Benth.) Maiden & Blakely, and the understory is often dominated by members of the family *Fabaceae,* such as *Pultanaea* spp. and *Acacia* spp. with some bare ground where canopy cover is dense. Other common species found are *Dodonaea viscosa* Jacq., *Astroloma* spp. *Pteridium esculentum* (G.Forst.) Cockayne, *Poa* spp. and *Hakea* spp. (Fig 5.3). *Leptospermum continentale* was located in the northern part of Mt. Bonython as a dense stand and *A. provincialis* less frequently as individual plants on adjacent hillsides. Both native vegetation sites were south facing with deep shaded areas with infestations of *R. anglocandicans* and *R. erythrops* adjacent to areas of uninvaded woodland (Fig. 5.3).

We introduced plants of *A. provincialis* and *L. continentale* purchased from ERA Nurseries into clumps of *R. anglocandicans* and *R. erythrops* plants where the latter had invaded native vegetation. At Mt. Bonython, fifteen *L. continentale* plants were planted with the two blackberry species, as well as fifteen *L. continentale* plants in adjacent open areas without blackberry, as a control. At the Greenhill Road Reserve, we planted fifteen *A. provincialis* plants within clumps of the two blackberries (*R. anglocandicans* and *R. erythrops*) and fifteen *A. provincialis* plants as a control planting in adjacent un-invaded areas of open ground.

Before planting, the two native species were weighed (with soil attached) and we recorded the weight, height and stem diameter 1cm above the soil surface. The field experiment ran over the same period as the shade house experiment for four and a half months through spring and summer 2013, beginning in September 2013 and ending in January 2014. In January, we recorded growth response parameters of height and stem diameter of *A. provincialis* and *L. continentale* plants at both field locations.

Data analysis

Linear models were used to explore relationships between the competition treatments and measured attributes of experimental plants from the field and shade house treatments. Analyses were conducted using the statistical packages R version 3.1.1 (2014-07-10) "Sock it to Me", stats ver. 3.1.1, R Core Team (2014), Applied spatial data analysis, Pebesma and Bivand (2005); ggplot2 ver.1.0.0, Wickham (2009); vegan ver.2.1-10, Oksanen *et al.* (2013); dplyr ver.0.2, Wickham and Francois (2014).

For the characteristics of stem area and plant height, initial values could be measured on individual plants and these were used as covariates in comparisons of average final measured values among treatments. Covariates and interactions involving covariates were not retained in the final models if they did not contribute significantly to the model predictions (Crawley, 2013). Pre-planned contrasts were conducted to compare the values of growth parameters between each of the competition treatment groups (native plants planted with a blackberry species) and the control groups (native species planted on their own).

Figure 5.3 Images of the field sites in the Mt. Lofty Ranges (winter) showing *R. anglocandicans* (A) and *R. erythrops* (B) invading native vegetation. *Rubus anglocandicans* (A) occupies the gully and almost completely excludes understory taxa compared to *R. erythrops* (B) where several native species are growing through the carpet-forming blackberry

Results

Shade-house competition- growth response of L. continentale

Comparisons of the means (App. Table 5.18 & 5.20) of six growth parameters (stem area, height, root wet and dry weights, leaf and stem wet and dry weights) for *L. continentale* using ANOVA or ANCOVA (Fig 5.4, App. Tables A5.1-A5.12) showed that growth was significantly reduced in at least one of the blackberry treatments, relative to the controls, for all parameters except root dry weight.

Pre-planned contrasts between the controls (*L. continentale* alone) and the three blackberry species treatments showed that *R. anglocandicans* caused the greatest reduction in the measured growth measures, followed by *R. erythrops* and then *R. leucostachys* (Fig. 5.4 a, b, c, d and f). The growth parameters of height and leaf and stem wet and dry weights were significantly reduced by all three blackberry species.

Shade-house competition-growth response of A. provincialis

In contrast to the results for *L. continentale*, comparisons of the means (App. Table 5.19 & 5.21) of the six growth parameters between control and blackberry treatments for *A. provincialis* (Fig 5.4, App. Tables A5.13-A5.17) show no significant differences. Overall, these results demonstrate that the growth of *L. continentale* plants was more affected by the presence of blackberries than was the growth of *A. provincialis* plants (Figs. 5.4, 5.5).

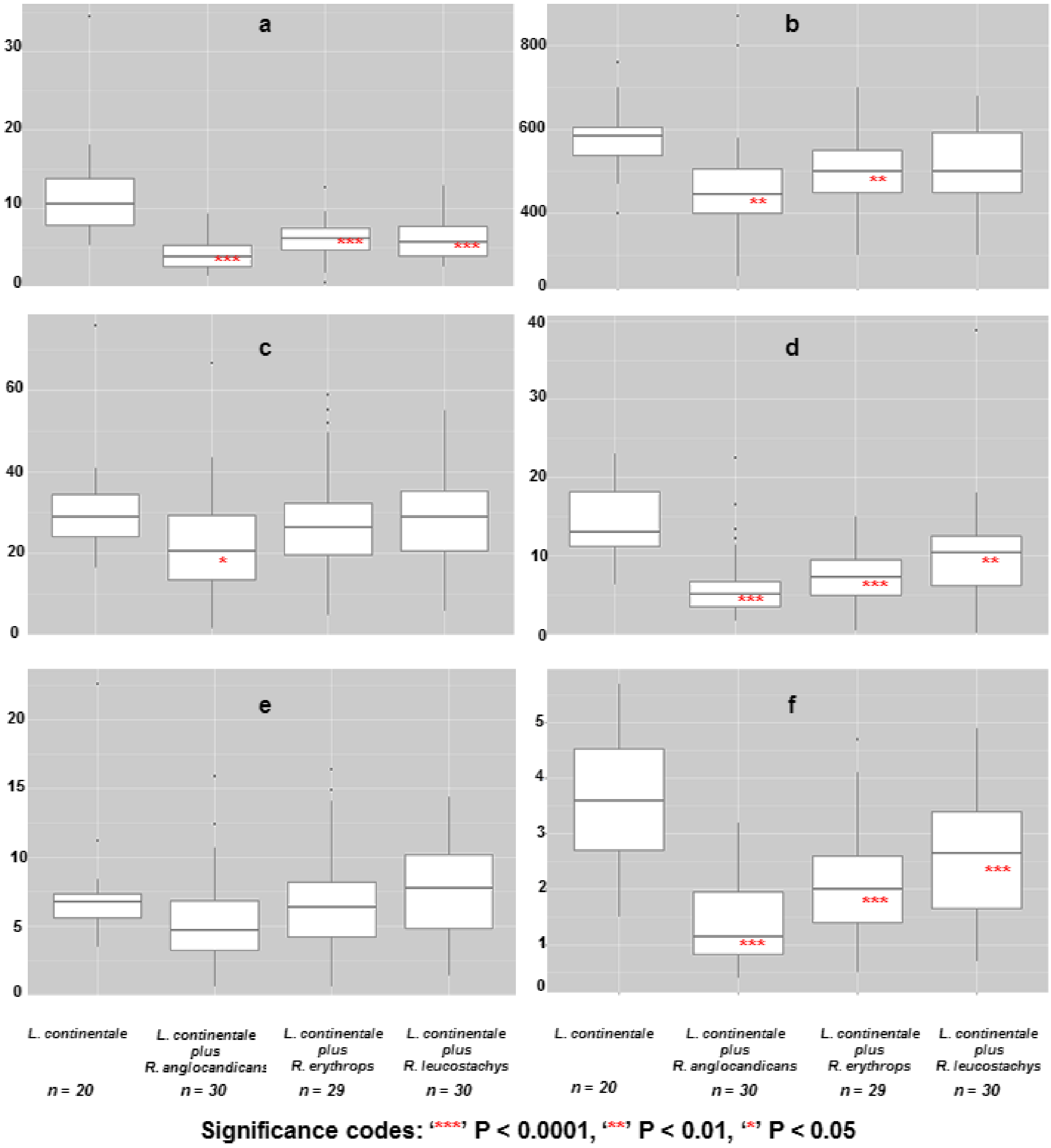
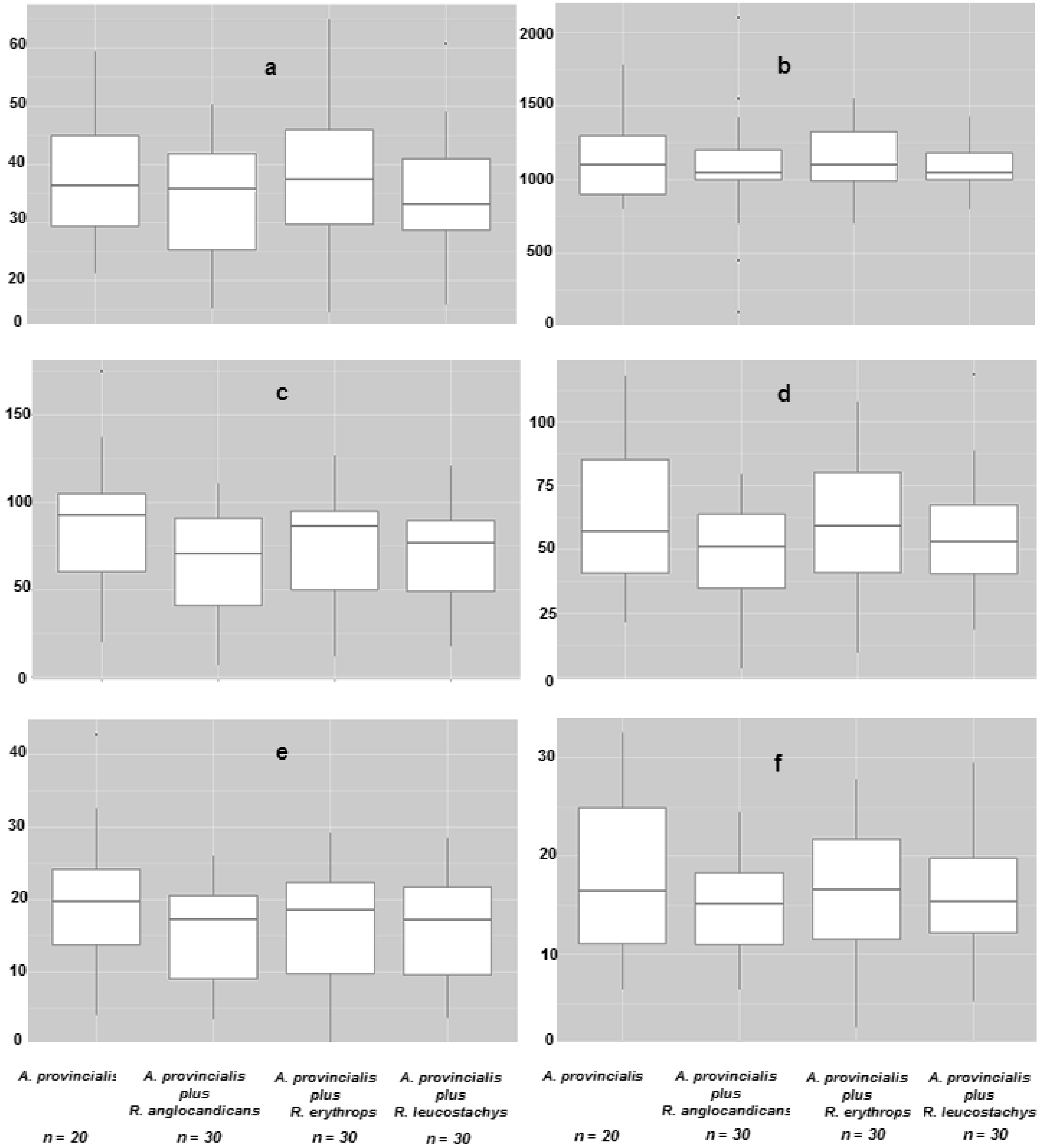


Figure 5.4 Summary plots of the growth response of *L. continentale (n = 20)* (shade-house conditions) planted on its own and with three blackberry species. (note [1] plant death reduction). Significant effects on growth were measured by one-way ANOVA contrasting the paired plantings of *L. continentale* with each blackberry species. The Y- axis represents the change in total growth response measured as **a** stem area growth (sq. mm) , **b** growth in height (mm), **c** roots growth, wet weight (g), **d** growth of leaves and stems, wet weight (g), **e** roots growth, dry weight (g), and **f** growth of leaves and stems, dry weight (g)



Significance codes: '*' P < 0.0001, '**' P < 0.01, '*' P < 0.05**

Figure 5.5 Summary plots of the growth response of *A. provincialis (n = 20)* (shade-house conditions) planted on its own and with three blackberry species. No significant effect on growth was measured for *A. provincialis* for the paired plantings. *Rubus anglocandicans (n = 30), R. erythrops (n = 30)* and *R. leucostachys (n = 30).* The Y-axis represents the change in total growth response measured as **a** stem area (sq. mm), **b** height (mm), **c** roots, wet weight (g), **d** leaves and stems, wet weight (g)**, e** roots, dry weight (g)**,** and **f** leaves and stems, dry weight (g)

Field competition

No significant competitive effects were measured in the field experiment in which fifteen plants of each of the native species *A. provincialis* and *L. continentale* were planted within clumps of blackberries compared with plants in the control group in adjacent open ground (Figs. 5.6, 5.7, App. Figs 5.22-5.29). Results for *A. provincialis* were inconclusive and are likely to be unreliable, as approximately half the experimental plants were browsed to ground level by kangaroos. *L. continentale* plants though were not affected by grazing. Summary plots of the effects on growth of *L. continentale* (Fig. 5.6, final stem area and final height) and *A. provincialis* (Fig. 5.6, final stem area and final height) in the field show non-significant effects of the blackberry treatments for both native species.

Overall, the results of field treatments involving both native species and two blackberry species were inconclusive.

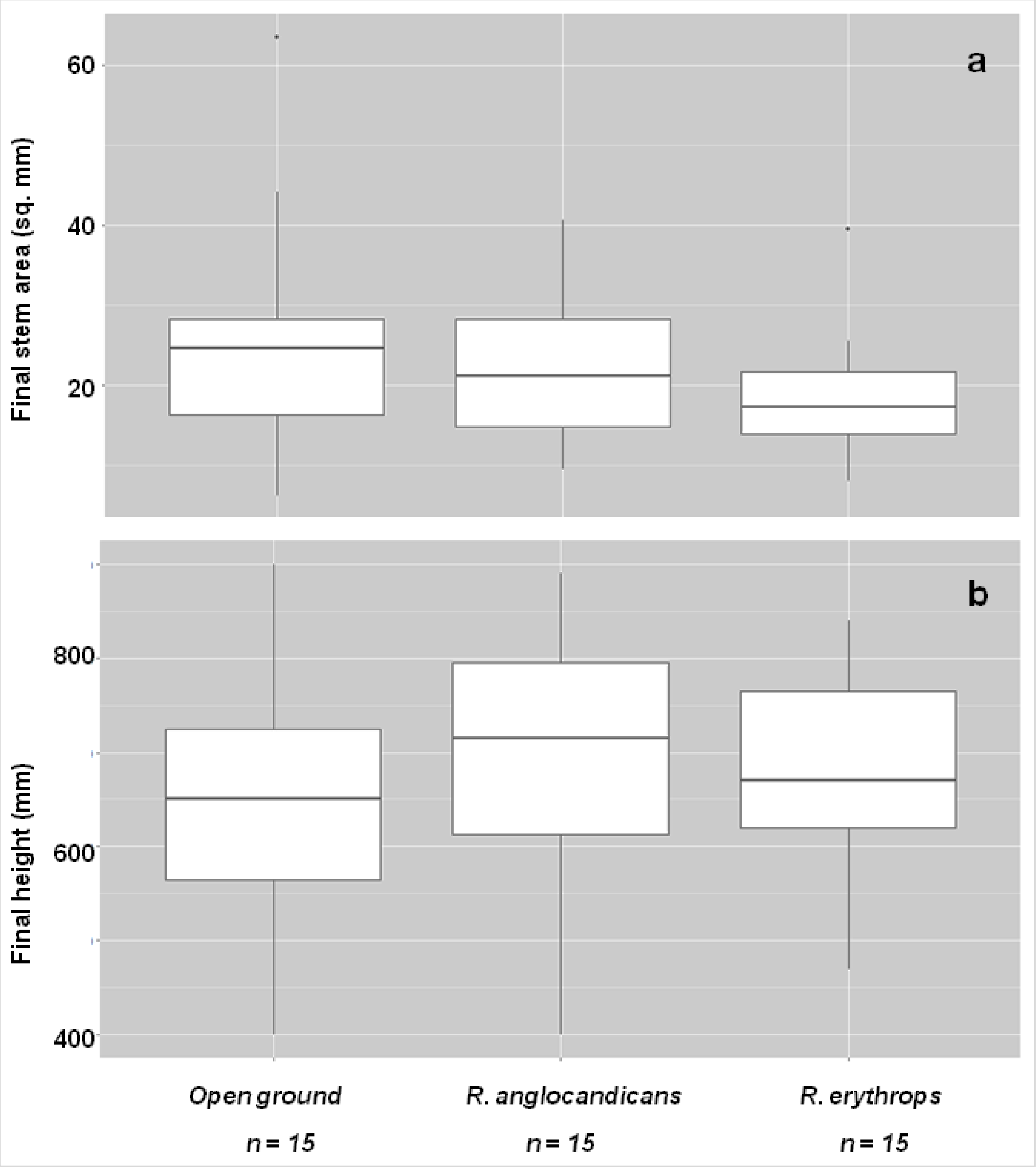


Figure 5.6 Summary plots of the growth of *L. continentale* (**a** final stem area and **b** final height) planted in the field in open ground, and planted within clumps of *R. anglocandicans* and *R. erythrops.* No significant growth responses were measured

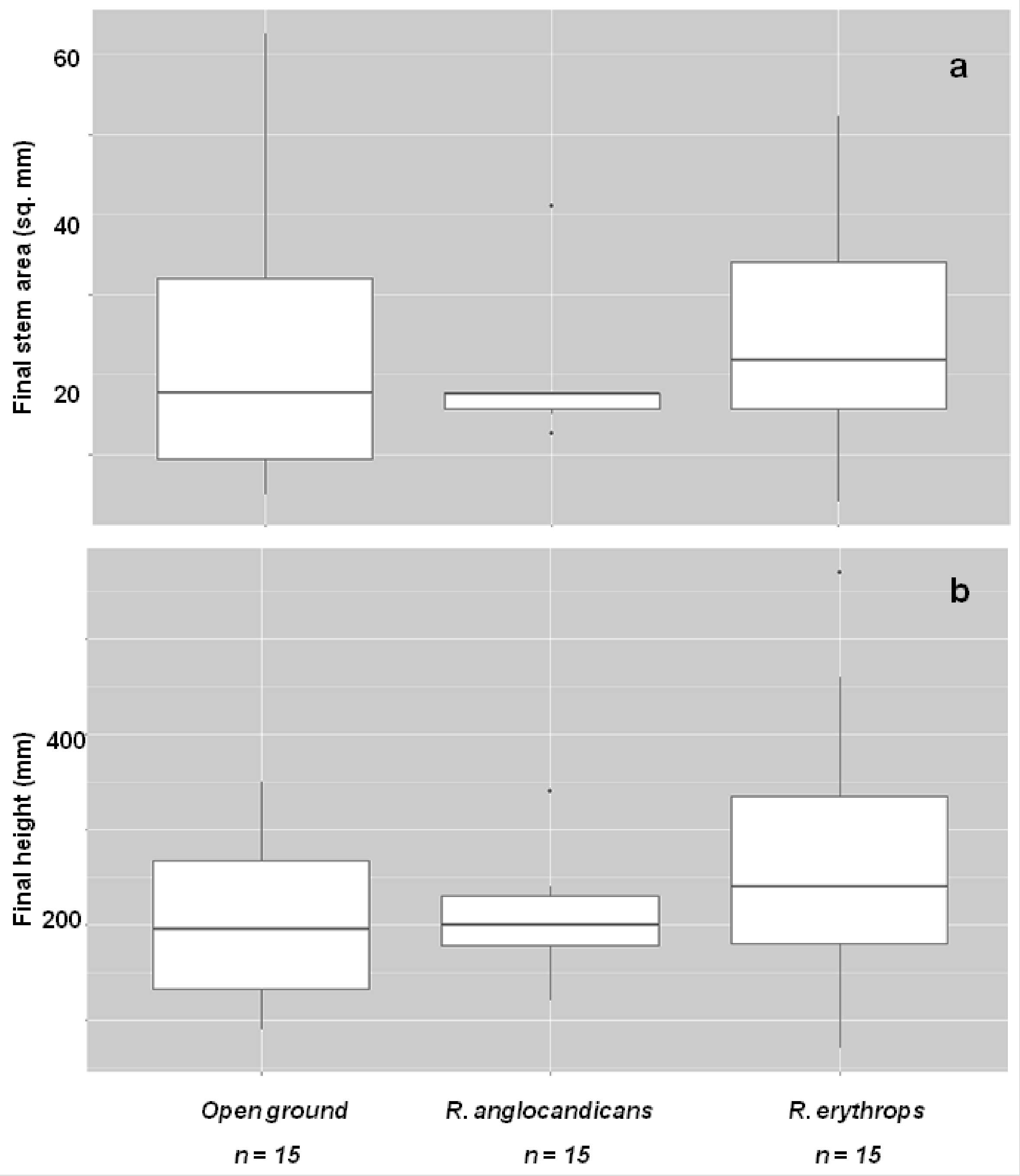


Figure 5.7 Summary plots of the growth of *A. provincialis* (a final stem area and b final height) planted in the field, in open ground, and planted within clumps of *R. anglocandicans* and *R. erythrops*. No significant growth responses were measured

Discussion

If blackberries were competing with the native species, we would expect the presence of blackberries to reduce the growth and vigour of the two target native species in both the field and shade house experiments. The analysis of the results from the field experiment was hampered by grazing on *A. provincialis* (presumably by kangaroos) and both *A. provincialis* and *L. continentale* displayed no significant reduction in growth when planted with blackberries compared to the control group planted in open ground. Analysis of the data from the shade house experiment shows the growth of *A. provincialis* was not significantly affected by co-potted blackberry species for both the above-ground and below-ground growth parameters measured for *A. provincialis* (Fig 5.5).

In contrast in the shade house experiment, *L. continentale* was significantly but differentially affected in its growth and vigour by all blackberry species suggesting this native plant is a poor competitor with invasive blackberries.

The habit of the two native species appears relatively similar in the shade house experiment in that both are upright in growth form over the period of the experiment (Fig. 5.1).

The growth of *L. continentale* (height and dry weight of above ground material) was significantly negatively reduced when in competition with blackberries in the pot experiment but the growth of *A. provincialis* was not significantly reduced by the end of the experiment. Controls of *L. continentale* (Fig. 5.4), grown on their own had mean values of 571.25mm for height (a relative difference of 451% in height over time) and 3.7g for the above ground vegetation dry weight, whereas *A. provincialis* (Fig. 5.5) had mean values of 1129mm for height (a relative difference of 584% in height over time) and 17.28g, for the above ground vegetation dry weight. While there were some differences in growth form between the plants of the two native species in the glasshouse, the different growth response of these two native species when paired with blackberry plants may also be due to other traits that could influence competition, such as differences in nutrient acquisition and utilisation.

Our field additive experiments did not provide a clear understanding of the competitive behaviour of the two native species (*A. provincialis* and *L. continentale*) planted with two blackberry species (*R. anglocandicans* and *R. erythrops*) under field conditions. This may be due to confounding factors like herbivory, but may also be due to the planting density of native species with blackberries. Notably, plant-animal interactions may play an important part in plant-plant interactions where animal herbivory competitively disadvantages some native species over invasive taxa (Cappuccino & Carpenter, 2005; Briske, 2007). In native plant communities in Australia, animal-plant interaction may in fact advantage invasive taxa in communities with low biodiversity by removal of seemingly effective competitor species such as members of the *Fabaceae.* However, this hypothesis has been little studied in Australia.

As plant-plant competitive interactions are complex and no single trait may advantage one species over another, we now suggest how some traits may influence the invasive behaviour to explain why some taxa are competitive and others are not.

Competition for light

Competition for light was reported by Downey and Smith (2000) as significant in limiting the introduction and spread of Scotch Broom (*C. scoparius*) in the Barrington Tops National Park, New South Wales Australia, but it is likely to be only one factor that influences invasion success. Although no similar studies are available for Australian *Rubus*, direct competition for light appears as one of the important factors in the invasion success of introduced *Rubus* taxa over native *Rubus* species in the Pacific north-west of the United States (McDowell, 2002; Caplan & Yeakley, 2006). The exotic *Rubus* taxa displayed not only vigorous growth but also excluded native *Rubus* with the former having significantly higher photosynthetic rates than that of the native *Rubus* taxa. Elements of functionally superior physiological traits inherent in invasive *Rubus* taxa, such as the rate of photosynthesis may work in conjunction with other morphological traits such as extended tolerances to climatic extremes in new ranges compared to their native ranges (McDowell, 2002; McDowell & Turner, 2002; Lambrecht-McDowell & Radosevich, 2005; Caplan & Yeakley, 2013).

A correlative study of the invasive blackberry *Rubus niveus* Thunb. found a significant effect of this species on plant cover and growth in most categories of Scalesia forest, Santa Cruz Island in the Galapagos (Renteria *et al.*, 2012). The authors found a significant decline in most introduced and native species richness, which they correlated to the competitive behaviour of *R. niveus* since its introduction in 1998. Dominance was attributed to the correlation of the density of *R. niveus* plants with the proportion of light reaching the understory within the forest community (Renteria *et al.*, 2012). Some vascular plant taxa responded differently to the presence of *R. niveus* and the overall effect showed a significant lower species richness and cover correlated with the percentage cover of *R. niveus*. Several introduced and native species though increased their presence under the lower light regimes suggesting more complex plant-plant interactions may be important in defining the impact on bio-diversity by *R. niveus* in Galapagos' forest communities (Renteria *et al.*, 2012).

Competition for light appears to be one of the factors that may have influenced the tolerance of *A. provincialis* to the presence of blackberries in our study. *Acacia provincialis* plants exhibited rapid growth over the four-month period and quickly overtopped all blackberry species and this species was not significantly affected by the blackberry treatments under shade house conditions (with 50% light reduction) for the growth parameters measured (Fig. 5.1). By comparison, the above ground vigour of *L. continentale* was significantly affected by all blackberry species but not equally so. *R. anglocandicans* displayed the greatest significant effect on the growth of *L. continentale*. The differences in the effects on the growth of *L. continentale* for each of the blackberry treatments could perhaps be explained by the greater upright growth habit and vigour of *R. anglocandicans* which in the field is characterised by rapid growth of canes and clumps that can exceed 3 metres in height in mature plants compared to the much lower growth form (1-2 metres in height) of the other two blackberry species. While these patterns of above-ground differences are consistent with competition for light, factors such as competition for other resources, e.g. soil nutrients, may also be important, particularly for plants grown in pots.

Are Australian native species providing protection to native plant communities?

Another functional trait may also explain the different competitive abilities apparent between the two native species in paired plantings with blackberry taxa. As some *Acacia* species are known as invasive species in introduced ranges, the nitrogen-fixing attributes of Australian *Acacias* (Yelenik *et al.*, 2004; Roura-Pascual *et al.*, 2009) may contribute to the ability of these species to compete with some blackberry species. Further investigation is needed to test whether nitrogen-fixing taxa (that are common in native vegetation communities within the Mt. Lofty Ranges) are effective competitors of *R. fruticosus* agg. species.

Vilà & Weiner, (2004), concluded that the generality that invaders are better competitors than natives needed to be further rigorously tested with combinations of individual native and invasive taxa. More generally, the hypothesis as to whether some native plant taxa in their natural ranges act as “resilient competitors” against invading plant taxa has not been fully tested. A meta-analysis by Van Kleunen *et al.* (2010) found that the majority of studies (117 field and garden studies) tested the links between performance-related traits of invaders in their invasive ranges and their success and not the contrary position of testing the performance of native plant taxa against invasive taxa. A review by Daehler (2003), based upon 79 independent native-invasive plant comparisons, found invaders were not statistically more likely to be successful in their new habitats than native competitors but rather success was dependent on growing conditions.

In Australian communities, further pair-wise trials both in garden and the field are needed to test whether taxa with performance-related traits, e.g. nitrogen-fixing taxa (that are common in native vegetation communities, within the Mt. Lofty Ranges) are effective competitors against *R. fruticosus* agg. species.

Concluding remarks

Competition among species in plant communities may influence the rate of invasion by introduced species. We tested whether invasive blackberry species are better competitors than native plant species and found mixed results using controlled shade house conditions and additive field experiments.

Results from the shade house group indicate the two native species performed differently under the three blackberry treatments. The biotic response (increase in growth) of *A. provincialis* was not restricted by *R. anglocandicans*, *R. erythrops* and *R. leucostachys*, whereas all blackberries significantly affected the growth of *L. continentale* suggesting it was a poor competitor.

Further manipulative studies are required to examine the performance of native taxa to the presence of individual blackberry taxa both in Australian native communities and in a controlled environment to test whether more bio-diverse communities are better protected from invasive blackberries.

Acknowledgements

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Appendix (App.) Chapter 5

Summary of model outputs

The appendix contains the statistical summary tables for the measured growth responses of two native species, *L. continentale* and *A. provincialis* grown in combination with three blackberry species in the shade house experiment (Tables A5.1-A5-17) and in the field experiment (Tables A5.22-A5.29).

Initial growth measurements recorded at the beginning of the blackberry treatments for *L. continentale* and *A. provincialis* are found in Tables A5-19 & A5.19. The final growth measurements of growth response for *L. continentale* and *A. provincialis* planted with three blackberry treatments are found in Tables A5.20 & A5.21.

Table A5.1 Summary ANOVA of the growth response (stem area) of *L. continentale* against three blackberry species (shade house conditions)

	d.f.	**SS**	**Mean sq.**	**F value**	**Pr(>F)**
Species	3	615.9	205.3	11.66	1.17e-06
Residuals	105	1847.9	17.6		

d.f. = degrees of freedom, SS = sum of squares

Table A5.2 Summary of contrasts of the linear model of the growth response (stem area) of *L. continentale* against three blackberry species (shade house conditions)

	Estimate	**Std. error**	**t value**	**Pr(>t)**
(Intercept)	11.7755	0.9381	12.553	< 2e-16
R. anglocandicans	-6.7408	1.2110	-5.566	2.01e-07
R. erythrops	-5.6754	1.2194	-4.654	9.53e-06
R. leucostachys	-5.6441	1.2110	-4.661	9.30e-06

Residual standard error: 4.195 on 105 degrees of freedom
Multiple R-squared: 0.25, Adjusted R-squared: 0.2285
F-statistic: 11.66 on 3 and 105 d.f., p-value: 1.17e-06

Table A5.3 Summary ANOVA of the growth response (height) of *L. continentale* against three blackberry treatments (shade house conditions)

	d.f.	**SS**	**Mean sq.**	**F value**	**Pr(>F)**
Species	3	149709	49903	4.48	0.005315
Residuals	105	1169535	11138		

d.f. = degrees of freedom, SS = sum of squares

Table A5.4 Summary of contrasts of the linear model of the growth response (height) of *L. continentale* against three blackberry species (shade house conditions)

	Estimate	**Std. error**	**t value**	**Pr(>t)**
(Intercept)	571.25	23.60	24.206	< 2e-16
R. anglocandicans	-110.68	30.47	-3.633	0.000435
R. erythrops	-76.77	30.68	-2.503	0.013873
R. leucostachys	-64.42	30.47	-2.114	0.036853

Residual standard error: 105.5 on 105 degrees of freedom
Multiple R-squared: 0.1135, Adjusted R-squared: 0.08815
F-statistic: 4.48 on 3 and 105 d.f., p-value: 0.005315

Table A5.5 Summary ANOVA of the growth response (roots wet weight) of *L. continentale* against three blackberry species (shade house conditions)

	d.f.	SS	Mean sq.	F value	Pr(>F)
Species	3	1048	349.4	1.995	0.1192
Residuals	105	18391	175.2		

d.f. = degrees of freedom, SS = sum of squares

Table A5.6 Summary of contrasts of the linear model of the growth response (roots wet weight) of *L. continentale* against three blackberry treatments (shade house conditions)

	Estimate	Std. error	t value	Pr(>t)
(Intercept)	30.855	2.959	10.426	< 2e-16
R. anglocandicans	-8.669	3.820	-2.269	0.0253
R. erythrops	-3.396	3.847	-0.883	0.3793
R. leucostachys	-2.542	3.820	-0.665	0.5073

Residual standard error: 13.23 on 105 degrees of freedom
Multiple R-squared: 0.05393, Adjusted R-squared: 0.02689
F-statistic: 1.995 on 3 and 105 d.f., p-value: 0.1192

Table A5.7 Summary ANOVA of the growth response (leaves and stems wet weight) of *L. continentale* against three blackberry species (shade house conditions)

	d.f.	SS	Mean sq.	F value	Pr(>F)
Species	3	911.6	303.86	10.96	2.551e-06
Residuals	105	2911.8	27.73		

d.f. = degrees of freedom, SS = sum of squares

Table A5.8 Summary of contrasts of the linear model of the growth response (leaves and stems wet weight) of *L. continentale* against three blackberry treatments (shade house conditions)

	Estimate	Std. error	t value	Pr(>t)
(Intercept)	14.415	1.178	12.242	< 2e-16
R. anglocandicans	-8.152	1.520	-5.362	4.92e-07
R. erythrops	-7.055	1.531	-4.609	1.14e-05
R. leucostachys	-4.545	1.520	-2.990	0.00348

Residual standard error: 5.266 on 105 degrees of freedom
Multiple R-squared: 0.2384, Adjusted R-squared: 0.2167
F-statistic: 10.96 on 3 and 105 d.f., p-value: 2.551e-06

Table A5.9 Summary ANOVA of the growth response (roots dry weight) of *L. continentale* against three blackberry species (shade house conditions)

	d.f.	**SS**	**Mean sq.**	**F value**	**Pr(>F)**
Species	3	72	23.99	1.737	0.1639
Residuals	105	1450	13.81		

d.f. = degrees of freedom, SS = sum of squares

Table A5.10 Summary of contrasts of the linear model of growth response (roots dry weight) of *L. continentale* against three blackberry treatments (shade house conditions)

	Estimate	**Std. error**	**t value**	**Pr(>t)**
(Intercept)	7.2400	0.8309	8.714	4.64e-14
R. anglocandicans	-1.9300	1.0726	-1.799	0.0748
R. erythrops	-0.3814	1.0800	0.353	0.7247
R. leucostachys	-0.0100	1.0726	-0.009	0.9926

Residual standard error: 3.716 on 105 degrees of freedom
Multiple R-squared: 0.04729, Adjusted R-squared: 0.02007
F-statistic: 1.737 on 3 and 105 d.f., p-value: 0.1639

Table A5.11 Summary ANOVA of the growth response (leaves and stems dry weight) of *L. continentale* against three blackberry species (shade house conditions)

	d.f.	**SS**	**Mean sq.**	**F value**	**Pr(>F)**
Species	3	63.49	21.164	18.95	6.751e-10
Residuals	105	117.26	1.117		

d.f. = degrees of freedom, SS = sum of squares

Table A5.12 Summary of contrasts of the linear model of the growth response (leaves and stems dry weight) of *L. continentale* against three blackberry species (shade house conditions)

	Estimate	**Std. error**	**t value**	**Pr(>t)**
(Intercept)	3.7000	0.2363	15.658	< 2e-16
R. anglocandicans	-2.2567	0.3051	-7.397	3.56e-11
R. erythrops	-1.5483	0.3072	-5.041	1.95e-06
R. leucostachys	-1.1467	0.3051	-3.759	0.000281

Residual standard error: 1.057 on 105 degrees of freedom
Multiple R-squared: 0.3513, Adjusted R-squared: 0.3327
F-statistic: 18.95 on 3 and 105 d.f., p-value: 6.751e -10

Table A5.13 Summary ANOVA of the growth response (height) of *A. provincialis* against three blackberry species (shade house conditions)

	d.f.	**SS**	**Mean sq.**	**F value**	**Pr(>F)**
Species	3	131706	43902	0.731	0.536
Residuals	107	6423452	60032		

d.f. = degrees of freedom, SS = sum of squares

Table A5.14 Summary ANOVA of the growth response (roots wet weight) of *A. provincialis* against three blackberry species (shade house conditions)

	d.f.	**SS**	**Mean sq.**	**F value**	**Pr(>F)**
Species	3	5682	1894.0	1.996	0.119
Residuals	107	101519	948.8		

d.f. = degrees of freedom, SS = sum of squares

Table A5.15 Summary ANOVA of the growth response (leaves and stems wet weight) of *A. provincialis* against three blackberry species (shade house conditions)

	d.f.	**SS**	**Mean sq.**	**F value**	**Pr(>F)**
Species	3	2789	929.8	1.807	0.15
Residuals	107	55057	514.6		

d.f. = degrees of freedom, SS = sum of squares

Table A5.16 Summary ANOVA of the growth response (roots dry weight) of *A. provincialis* against three blackberry species (shade house conditions)

	d.f.	**SS**	**Mean sq.**	**F value**	**Pr(>F)**
Species	3	251	83.77	1.32	0.272
Residuals	107	6791	63.47		

d.f. = degrees of freedom, SS = sum of squares

Table A5.17 Summary ANOVA of the growth response (leaves and stems dry weight) of *A. provincialis* against blackberry treatments (shade house conditions)

	d.f.	**SS**	**Mean sq.**	**F value**	**Pr(>F)**
Species	3	88	29.31	0.765	0.516
Residuals	107	4098	38.30		

d.f. = degrees of freedom, SS = sum of squares

Table A5.18 Initial means and standard deviations of growth measurements at the beginning of the shade house experiment of *L. continentale* planted individually[1] and pair planted with three blackberry treatments[2]

	Initial Growth Measurements		
Treatments	**Stem area sq. mm**	**Height mm**	**Total Weight g**
***L. continentale*[1]**	4.15±1.56	25.80±4.64	79.55±5.27
***R. anglocandicans*[2]**	2.58±1.37	20.83±4.62	76.97±3.94
***R. erythrops*[2]**	3.79±1.40	23.72±4.63	77.97±3.10
***R. leucostachys*[2]**	4.05±1.55	22.83±3.10	78.88±4.03

Table A5.19 Initial means and standard deviations of growth measurements at the beginning of the shade house experiment of *A. provincialis* planted individually[3] and pair planted with three blackberry treatments[4]

	Initial Growth Measurements		
Treatments	**Stem area sq. mm**	**Height mm**	**Total Weight g**
***A. provincialis*[3]**	13.63±4.62	65.95±10.88	89.07±12.80
***R. anglocandicans*[4]**	11.71±3.96	66.63±7.23	82.56±6.80
***R. erythrops*[4]**	12.89±4.31	70.52±13.40	90.06±8.08
***R. leucostachys*[4]**	14.08±4.72	71.13±10.47	90.29±10.65

Table A5.20 Final means and standard deviations of the total growth measurements of *L. continentale* planted individually[1] and pair planted with three blackberry treatments[2] (shade house)

	Final Growth Measurements						
Treatments	**Stem area sq. mm**	**Height mm**	**Weight roots g (wet)**	**Weight above ground g (wet)**	**Combined weight g (wet)**	**Weight roots g (dry)**	**Weight above ground g (dry)**
***L. continentale*[1]**	11.78±6.53	571.25±90.12	30.86±12.36	14.42±4.61	45.27±15.71	7.24±4.00	3.70±1.25
***R. anglocandicans*[2]**	4.21±2.12	460.57±129.63	22.19±13.76	6.26±4.74	28.45±17.05	5.31±3.49	1.44±0.81
***R. erythrops*[2]**	6.10±2.54	494.48±92.68	27.46±14.36	7.36±3.78	34.82±17.07	6.86±3.97	2.15±1.08
***R. leucostachys*[2]**	6.13±2.71	506.83±99.55	28.31±12.07	9.87±7.09	38.18±17.55	7.23±3.48	2.55±1.11

Table A5.21 Final means and standard deviations of the total growth measurements of *A. provincialis* planted individually[3] and pair planted with three blackberry treatments[4] (shade house)

	Final Growth Measurements						
Treatments	**Stem area sq. mm**	**Height mm**	**Weight roots g (wet)**	**Weight above ground g (wet)**	**Combined weight g (wet)**	**Weight roots g (dry)**	**Weight above ground g (dry)**
***A. provincialis*[3]**	37.92±12.48	1129.00±259.61	87.19±38.45	63.07±27.31	150.26±64.34	19.74±9.55	17.28±7.52
***R. anglocandicans*[4]**	34.30±10.31	1063.33±330.76	66.55±27.22	50.43±18.75	116.98±43.67	15.43±7.11	14.95±5.12
***R. erythrops*[4]**	38.32±12.20	1144.03±221.69	75.92±29.90	61.11±23.26	137.03±49.96	16.03±8.15	16.78±6.43
***R. leucostachys*[4]**	34.07±10.33	1077.83±130.75	70.24±29.44	54.14±22.33	124.39±48.42	16.29±7.42	15.62±5.94

Table A5.22 Summary ANOVA of the growth response (height) of *L. continentale* against two blackberry species (field)

	d.f.	**SS**	**Mean sq.**	**F value**	**Pr(>F)**
Species	2	25796	12898	0.896	0.415
Residuals	49	705135	14391		

d.f. = degrees of freedom, SS = sum of squares

Table A5.23 Summary of contrasts of the linear model of the growth response (height) of *L. continentale* against two blackberry treatments (field)

	Estimate	**Std. error**	**t value**	**Pr(>t)**
(Intercept)	650	23.09	28.15	< 2e-16
R. anglocandicans	48.57	39.51	1.229	0.225
R. erythrops	38.18	42.91	0.890	0.378

Residual standard error: 120 on 49 degrees of freedom
Multiple R-squared: 0.03529, Adjusted R-squared: -0.004084
F-statistic: 0.8963 on 2 and 49 d.f., p-value: 0.415

Table A5.24 Summary ANOVA of the growth response (height) of *A. provincialis* against two blackberry species (field)

	d.f.	**SS**	**Mean sq.**	**F value**	**Pr(>F)**
Species	2	45627	22814	2.268	0.118
Residuals	36	362065	10057		

d.f. = degrees of freedom, SS = sum of squares

Table A5.25 Summary of contrasts of the linear model of the growth response (height) of *A. provincialis* against two blackberry treatments (field)

	Estimate	**Std. error**	**t value**	**Pr(>t)**
(Intercept)	198.5	21.38	9.269	4.53e-11
R. anglocandicans	13.48	46.19	0.292	0.2250
R. erythrops	78.18	37.03	2.111	0.0418

Residual standard error: 100.3 on 36 degrees of freedom
Multiple R-squared: 0.1119, Adjusted R-squared: 0.06258
F-statistic: 2.268 on 2 and 36 d.f., p-value: 0.118

Table A5.26 Summary ANOVA of the growth response (stem area) of *L. continentale* against two blackberry species (field)

	d.f.	SS	Mean sq.	F value	Pr(>F)
Species	2	254	127.2	1.148	0.326
Residuals	49	5430	110.8		

d.f. = degrees of freedom, SS = sum of squares

Table A5.27 Summary of contrasts of the linear model of the growth response (stem area) of *L. continentale* against two blackberry treatments (field)

	Estimate	Std. error	t value	Pr(>t)
(Intercept)	24.311	2.026	12.000	3.38e-16
R. anglocandicans	-1.996	3.467	-0.576	0.567
R. erythrops	-5.692	3.765	-1.512	0.137

Residual standard error: 10.53 on 49 degrees of freedom
Multiple R-squared: 0.04476, Adjusted R-squared: 0.005773
F-statistic: 1.148 on 2 and 49 d.f., p-value: 0.326

Table A5.28 Summary ANOVA of the growth response (stem area) of *A. provincialis* against two blackberry species (field)

	d.f.	SS	Mean sq.	F value	Pr(>F)
Species	2	15.1	7.53	0.134	0.875
Residuals	36	2019.4	56.10		

d.f. = degrees of freedom, SS = sum of squares

Table A5.29 Summary of contrasts of the linear model of the growth response (stem area) of *A. provincialis* against two blackberry treatments (field)

	Estimate	Std. error	t value	Pr(>t)
(Intercept)	16.5218	1.5968	10.347	2.48e-12
R. anglocandicans	-1.3647	3.4495	-0.396	0.695
R. erythrops	0.5991	2.7658	0.217	0.830

Residual standard error: 7.49 on 36 degrees of freedom
Multiple R-squared: 0.007401,, Adjusted R-squared: -0.04774
F-statistic: 0.1342 on 2 and 36 d.f., p-value: 0.875

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Chapter 6

Chromosome numbers and genome size of weedy *Rubus fruticosus* L. aggregate in Australia

[1]John Marshall, [2] Molly A. Whalen, [3] Duncan A. Mackay

[1, 2, 3] Flinders University, School of Biological Sciences, GPO Box 2100, Adelaide, S.A. 5001, Australia.

Abstract

Background Life history traits associated with a complex breeding system are suggested to enhance invasion success in some plant groups. Polyploidisation, which may be associated with interspecific hybridisation among related taxa, and smaller genome size also appear to influence the invasive fitness and ecological adaptability of taxa in introduced ranges in some plant groups. Closely related *R. fruticosus* L. agg. taxa originating from Europe have become some of Australia's worst weeds but current knowledge of the cytology of taxa in their invasive range in Australia and of the possible occurrence of interspecific hybridisation in this region is very limited.

Aims Using flow cytometry and chromosome counts, our aim was to assess chromosome numbers and variation in nuclear DNA content for six species in their invasive range within the Mt. Lofty Ranges in Australia and to assess whether there is any evidence from estimates of nuclear DNA content that diploid and polyploid species are hybridising in this region.

Results Based on chromosome numbers from Australian material, all but one of the six exotic blackberry taxa was tetraploid in the invasive range in Australia, with the exception being the occurrence of the diploid species *Rubus ulmifolius.* Results from both chromosome counts and flow cytometry for these six taxa in Australia are generally consistent with available information on ploidy level for these taxa based on material collected in their native ranges in Europe and Britain with the exception of *R. anglocandicans* for which confirmed chromosome numbers from its native range are lacking. Thus, there is no evidence of a change in ploidy level in the introduced ranges of these taxa. Nuclear genome size of the five tetraploid blackberry taxa was small and varied over a tight range.

In the survey of chromosome numbers and nuclear DNA size, no evidence was detected of higher ploidy levels or triploid cytotypes in populations. One way in which the latter could arise would be interspecific hybridisation between related diploid and tetraploid species of blackberries in the Mt. Lofty Ranges.

However, the absence in the survey of higher ploidy levels or triploid cytotypes suggests no hybridisation has occurred between related species of blackberries in the Mt. Lofty Ranges.

Main conclusions Reliable estimates of nuclear DNA size from flow cytometry linked with chromosome counts can provide valuable information on ploidy levels of closely related problem weed species in their introduced ranges. Five of the six exotic blackberry taxa assessed in their invasive range in Australia were tetraploid and one was a diploid, and there was no evidence of a change in ploidy level in the introduced ranges of these taxa relative to their native range. No evidence of interspecific hybridisation between diploid and tetraploid taxa was observed in the Mt. Lofty region. in the limited sample of *Rubus* taxa. The six taxa displayed relatively small genome sizes. It has been suggested that small genome sizes (below 10 pg) is a feature of many weeds. Importantly, specific biological and ecological information allows land managers and researchers to understand more fully the linkages between different aspects of their biology and the trait of weediness in blackberries introduced in the Mt. Lofty Ranges.

Keywords C- values, genome size, ploidy, invasion, *Rubus fruticosus*, flow cytometry, chromosome numbers, weeds

Introduction

Exotic blackberries of the *R. fruticosus* L. agg. were introduced into Australia during colonial settlement and are now one of Australia's worst weeds (Department of Agriculture Fisheries and Forestry, 2012). However, knowledge of the traits and mechanisms, including the breeding system, which make these species so invasive in Australia, is limited (French *et al.*, 2014).

Blackberries in Australia

In Australia about 15 species of the *R. fruticosus* agg. have been introduced. Species such as *Rubus anglocandicans* A. Newton, which has been identified as the most widespread species in Australia, appear strongly clonal (by both vegetative spread and production of clonal seed) (Evans & Weber, 2003; Evans *et al.*, 2007). Very little work though has been undertaken to examine many aspects of the biology of individual blackberry species in Australia, including their cytology and the status of their breeding system, or to examine whether in fact there is evidence that taxa are hybridising in areas where they co-occur (Evans & Weber, 2003; French *et al.*, 2014).

In South Australia, 10 of the ca. 15 species known to occur across Australia are present in the Mt. Lofty Ranges, including *Rubus ulmifolius* Schott var. *ulmifolius*, a taxon that can be found under cultivation in Europe (Hammer *et al.*, 2004). *Rubus ulmifolius* is considered to be the only fully sexually reproducing species of the *R. fruticosus* agg. in Australia (Evans *et al.*, 2007) and is potentially a progenitor species important in the development of hybrids with other members of the *R. fruticosus* agg. One condition relevant to the possibility of interspecific hybridisation among species is that 10 species occur in the introduced range of the *R. fruticosus* agg. in the Mt. Lofty Ranges, South Australia (see collections at the State Herbarium of South Australia (AD).

Blackberry reproduction, ecology and biology

Blackberries are members of the family *Rosaceae* and many blackberry species have a complex breeding system as do members of other genera such as *Crataegus*, *Taraxacum,* and *Hieracium.* Some species of these genera are known as agamo-species reproducing through a process known as agamospermy, generating viable seed in the maternal line without gamete reduction (Judd *et al.,* 2002, pg.147). The majority of species in the *R. fruticosus* agg. are polyploids and based on studies in Europe are thought to produce seed largely through asexual processes (apomixis), although sexual reproduction may occasionally occur, e.g.

because of interspecific hybridisation (Edees & Newton, 1988; Nybom, 1988, 1995; Evans *et al.*, 2004).

The dominant mode of reproduction in many species is likely to be through a method of tip rooting of primocane apices in autumn and winter (Watson, 1958; Amor, 1974). Vegetative spread is characterised by a radial expansion pattern of primocanes, allowing a rapid colonisation of suitable habitats particularly in woodland margins, along streamlines and in forest gaps (Watson, 1958; Amor, 1974; Taylor, 2005). Blackberries are adapted to a diversity of habitats in both their native and introduced ranges worldwide, ranging from the Arctic Circle to the tropics (Clark *et al.*, 2007).

Historic studies in Europe and Great Britain suggest that *Rubus* subgen. *Rubus*, has in excess of 2500 agamospecies identified by morphological studies (Edees & Newton, 1988). More generally, agamospecies remain mostly reproductively isolated and groups with agamospecies often contain some sexual taxa that hybridise, thereby allowing potential new hybrid progeny to form (Judd *et al.*, 2002, pg.147). This event though has not commonly been detected in nature although intra- and inter-specific crosses of indigenous *R. fruticosus agg.* species in Europe have been demonstrated with just a few European species that resulted in approximately 10% sexually derived progeny through pollination of unreduced and reduced egg cells (Werlemark & Nybom, 2003).

The majority of knowledge of the range of ploidy level variation among *Rubus* (Rosaceae) species is summarised in Thompson (1997). The basic chromosome number in *Rubus* is seven and species with ploidy levels up to *18x* were recorded based predominantly on previous studies from *Rubus* collected in their native ranges in North America, Europe and Great Britain (see Thompson, 1997 and references within). The most common ploidy level among individual species of European blackberries appears to be *4x* (tetraploid) as reported by Thompson (1997) and in a small number of studies of European taxa in their native range (Krahulcová, 1997; Krahulcová & Holub, 1998; Krahulcová *et al.*, 2013).

Introduced Rubus *taxa and ploidy, breeding system level and hybridisation*

Polyploidy, hybridisation and the ability to reproduce asexually can all potentially influence invasiveness of plants (Ellstrand & Schierenbeck, 2000; Ellstrand, 2009). For instance, the ability to reproduce asexually can facilitate colonisation by plants. An example of this has been found in the invasive species *R. alceifolius* on La Reunion Island in the Indian Ocean (Amsellem *et al.*, 2000). Amsellem *et al.* (2001a) suggested that *R. alceifolius* has shown a significant shift from sexually produced seed in its native range in South East Asia toward an apomictic (asexual) mode of seed production in its introduced range in Madagascar and La Réunion Island and that this shift may be related to hybridisation between introduced *R. alceifolius* and native populations of *Rubus roridus* Lindl., in Madagascar. They also investigated the possibility of an increase in the ploidy level in *R. alceifolius,* on La Reunion Island and Madagascar relative to its native range in Asia using flow cytometry but did not find an increase in ploidy level in populations in its introduced range (Amsellem *et al.*, 2001b).

In an invasion context, the process of hybridisation can but does not always result in invasive new hybrid progeny. Clark and Jasieniuk (2012) detected spontaneous hybrids from sexual reproduction between native *Rubus* species and exotic *Rubus* species introduced into the western United States, as did Randell *et al.* (2004) in Hawai'i but these authors did not consider the hybrid progeny currently to be a serious ecological threat in their respective ranges.

Flow cytometry

Flow cytometry in biology involves the analysis of biological material through the detection of the light-absorbing or fluorescing properties of particles and cells, or of sub-cellular preparations such as chromosomes, as they pass in a narrow stream through a laser beam (Shapiro, 2003, pg. 172).

Flow cytometry has been used in a variety of studies of the genus *Rubus*. As noted above, flow cytometry was used to assess ploidy levels in the native and invasive ranges of an invasive *Rubus* species (Amsellem *et al.*, 2001b). Recent studies on *Rubus* species in their native range and horticultural varieties have employed flow cytometry methods as a rapid reliable method of determining nuclear DNA size and estimating ploidy level (Meng & Finn, 2002; Šarhanová *et al.*, 2012; Krahulcová *et al.*, 2013).

For example, a recent field study by Šarhanová *et al.* (2012) using both FCM and flow cytometry seed screen (FCSS), a derivative technique in flow cytometry, demonstrated variability in the reproductive modes in different diploid, triploid and tetraploid species of *Rubus* subgen. *Rubus* in their native range of Eastern Europe. They found some progeny were sexually derived and that approximately 17 species or sub-species had triploid genomes. Other studies of native *Rubus* in Europe have incorporated FCM to understand the extent and distribution of cytological variation in taxa. For example, Ryde (2010) used FCM in conjunction with molecular techniques to understand the spatial distribution of genetic variation in *Rubus vikensis* A. Petersen ex. G. Wendt., which occupies a variety of habitats but it was not known whether the disjunct populations represented a single species. FCM has also been employed to establish the nuclear DNA content (estimations of ploidy and genome size) of *Rubus chamaemorus* L., an economically important species rich in biologically active metabolites (Thiem & Sliwinska, 2003).

Flow cytometry has been used to study progeny in plants to assess if interspecific hybridisation between taxa of different ploidy levels may be occurring (Suda *et al.*, 2007). A good example of this is a study by Suda *et al.* (2010) who measured significant correlations in genome size differentiating new hybrids of closely related *Fallopia* taxa in Eastern Europe and suggested the technique was reliable for the identification of new invasive knotweed species. Given the variation in ploidy level in *Rubus*, flow cytometry can be a useful approach in studies examining potential hybridisation in this genus.

In flow cytometry methods, having standardised protocols for estimating the nuclear genomic size and ploidy of plant cells, is a basic requirement for obtaining repeatable results (Greilhuber, 2005). Standardisation not only includes the selection of appropriate plant standards in the analysis of unknown plant material but also is a best practise methodology approach in application that can account for potential methodologically driven intra-specific variation (Greilhuber, 2005). As an example, FCM variation measured in *Glycine max* Willd. (soybean) cultivars by some investigators (Graham *et al.*, 1994; Rayburn *et al.*, 1997), was considered to be induced by the methodology employed and not attributed to actual variation in the *G. max* genome (Greilhuber & Obermayer, 1997). For this reason, we provide detailed methods related to the literature on variation in FCM analysis.

Aims

We used flow cytometry methods and chromosome counts to investigate six invasive taxa of the *R. fruticosus* agg. in the Mt. Lofty Ranges, to assess chromosome numbers from plants in the invasive range in Australia and variation in nuclear genomic DNA size (if any) within these taxa in this region. We ask whether there is any evidence from estimates of nuclear genomic DNA size to suggest that hybridisation may have occurred between the diploid and polyploid species in this region.

Material and Methods

Plant material

We collected material of plants of *R. fruticosus* agg. species in the Mt. Lofty Ranges, South Australia from rooting tips of primary canes (primocanes) in June and July 2013. These were grown in pots under shade house conditions until required. We collected samples of five to ten ramets of spatially separated genets for *Rubus anglocandicans* A. Newton, *Rubus leucostachys* Schleich. ex Sm., *Rubus erythrops* Edees & A. Newton, *Rubus rubritinctus* W. C. R. Watson, *Rubus laciniatus* Willd. and *Rubus ulmifolius* Schott var. *ulmifolius*. Plants of *R. fruticosus* agg. species from which rooting tips of primocanes were collected in the field were identified (Barker & Barker, 2005), and voucher specimens of these plants were prepared and included in the herbarium collection at AD (App. Table A6.1). Primocanes were then potted (after washing in water to remove soil) using "coco-peat potting mix" (Waite Agricultural Research Institute). Potted primocanes were grown in a 50% light reduced shade house with a watering cycle of three minutes every six hours until rapid vegetative growth was established after 2 months.

Chromosome Counts

To provide sufficient good quality root tips for chromosome counts we grew blackberries under shade house conditions. Well-formed root tips were harvested in the late morning and immediately placed in iced water, washed to remove debris, then packed in ice and stored in a 4°C cold room for at least 48 hours (Singh, 2003).

All procedures were carried out in screw top Eppendorf® tubes. Incubations were performed in a heating block.

We prepared fresh fixative on the day of the examination of root tips (ethanol : acetic acid, ratio 3:1 and added a mordant of saturated ferric acetate (5% v/v, final) (Ahloowalia, 1965). The tip section (1.5-2mm) of the root was then added to fixative pre-heated for 5 minutes at 60^0 C and incubated for 30 minutes.

Root tips were then removed and placed in 60^0C 1M HCL, and incubated for a further 10-12 minutes. Root tips were rinsed in 70% ethanol for 2 minutes with several changes.

The root tips were then placed in a new tube and aceto-orcein chromosome stain added to cover the root-tips and incubated at 60^0C for 20-30 minutes (La Cour, 1941).

Stained chromosomes were prepared by macerating the root tips in room temperature 45% acetic acid and squashed on a pre-warmed glass slide (50°C).

Flow Cytometry

Histograms of counts of nuclei of blackberry species combined with *Pisum sativum* L. cv Lincoln as a standard were generated by a method modified from Galbraith and Lambert (2009) using an *Accuri™ C6 Cytometer*© and *Accuri™* CFlow Plus software. In our method, a two-step Otto's buffer system was used for preparation and staining of nuclei with propidium iodine (Otto, 1990). Buffers and solutions were routinely filtered through a 0.22µm filter before use and all preparation and procedures were performed on ice at 4 °C. We sampled new leaf material in the early morning and placed the leaf into room temperature demineralised water for more than two hours to ensure cells were turgid. Then, three to four square centimeters of leaf were chopped in 1.5mls of Otto 1 buffer (0.1 M citric acid, 0.5% Tween 20; Sigma© P2287) with a new razor blade on a new disposable petri dish, until a darker colour change occurred. We filtered this suspension through a BD Falcon® 40µm nylon cell strainer© (ref# 352340) and then centrifuged samples in a 1.5ml Eppendorf® tube at 150g for five minutes. The supernatant was then removed leaving approximately 50µl of liquid above the pellet. The tube was flick-mixed to resuspend the pellet and 2µg of RNase A (Sigma© R5125, Type 111A) from a 1mg/ml stock solution was added and the tube mixed again.

Samples were generally stable at 4 °C and gave good results if processed within 24 hours. Preparations of leaf samples that showed "browning of the nuclei suspension and/or opacity", gave poor nuclei yields because of antagonistic chemical release due to over-chopping (Greilhuber *et al.*, 2007, pg. 90). Leaf samples of this type were not used.

Before analysis in the *Accuri™ C6 Cytometer*©, 200µl of Otto 2 buffer (0.4 M $Na_2HPO_4 \cdot 12H_2O$, kept at room temperature), containing 50µg/ml of both propidium iodine (PI) (Sigma© P4170) and RNase A was added to each sample and incubated at room temperature for 5 minutes.

Samples were run in the *Accuri™ C6 Cytometer*© on medium fluidics (35µl/minute, 16µm core setting) with a back flush followed by 20 seconds of water between samples. The *Accuri™ C6 Cytometer*© data is digitally collected over a range of 7.2 decades equal to 16 million channels. The *Accuri™ C6 Cytometer*© was routinely calibrated against Spherotech® 6 and 8 bead calibration particles available from BD Biosciences® (App., Fig A6.1). Variation among geo-mean peaks for the calibration particles over time ranged from 4.7% to 4.93% *(n=15)*. *Rubus fruticosus* agg. taxa were calibrated against the internal standard, *Pisum sativum* L. cv Lincoln (Bennett & Leitch, 1995). *P. sativum* L. is recognised as a standard for flow cytometry with a value of 9.1picograms (pg) used here (Greilhuber & Ebert, 1994; Doležel *et al.*, 1998; Doležel & Bartoš, 2005) and enabled estimation of relative DNA size of six *Rubus* taxa.

Samples of blackberries that were run together with *P. sativum* L. as an internal standard included samples for three blackberry species - *R. anglocandicans, R. leucostachys* and *R. erythrops*. For samples of blackberries that were not run together with a *P. sativum* L.

standard, an ‘external standard’ of *P. sativum* L. was used in the estimation of the relative fluorescences of the blackberry peaks, consisting of data on the relative fluorescences of the samples of *P. sativum* L. that had been run with *R. anglocandicans, R. leucostachys* and *R. erythrops.*

Analysis using Flowing Software

Analysis was performed using *Flowing Software*$^{©}$ (Terho, 2013). All sample files were exported from the *Accuri™ C6 Cytometer*$^{©}$ as flow cytometry standard (FCS 3.0) format. Samples with low event numbers were discarded (less than 5000 total events). Using *Flowing Software*$^{©}$ we examined sample nuclei clouds in log scatter plots to determine the pattern of G1 nuclei that formed a tight line (known as the PI diagonal) in good quality samples. We manually gated the G1 peaks in log FL2 histograms of blackberry samples and the internal standard (*P. sativum* L.) and calculated the statistics for the position of relative fluorescence, median, geometric mean (GeoMean) and coefficient of variance (CV). Samples with CV percentages above 5% were discarded from further analysis because they were considered to be too variable. The nuclear DNA amount of each blackberry sample was calculated using the formula (Arumuganathan & Earle, 1991);

$$\textbf{Nuclear DNA amount} = \left(\frac{\textbf{Geometric mean of fluorescence of } \textbf{\textit{Rubus}} \textbf{ plant}}{\textbf{Geometric mean of florescence of } \textbf{\textit{P. sativum}} \textbf{ L.}} \right) \textbf{x 9.1 pg}$$

Results

Chromosome Counts

We estimated chromosome numbers in metaphase cells for Australian plants of six introduced *R. fruticosus* agg. taxa. Chromosome counts for each taxon were derived from multiple counts (Fig. 6.1, Table 6.1), and counts were made from root tips pooled from the plants indicated in Table A6.1. Exact counts of 2n=28 and approximate or *circa* counts of 2n=ca. 27 and *26* (from material for which it was not possible to make an exact count) were recorded for plants of five species of the *R. fruticosus* agg. from their introduced ranges in Australia, i.e. *R. anglocandicans*, *R. leucostachys*, *R. erythrops*, *R. rubritinctus*, and *R. laciniatus*. For one taxon *R. ulmifolius* Schott var. *ulmifolius*, counts of 2n=14 were recorded from Australian plants (Fig. 6.1, Table 6.1).

Table 6.1 Summary of chromosome counts for six species of the *R. fruticosus* agg. from Australian plant material with the number of cells counted for each taxon with exact counts and approximate or *circa* chromosome counts

Species	***Number of cells counted***	***Counts (2n)***	
		exact	***approximate***
R. anglocandicans	11	**28**(7)	ca. 27(4)
R. leucostachys	7	**28**(4)	ca. 27(3)
R. erythrops	11	**28**(6)	ca. 27(4) ca. 26(1)
R. rubritinctus	7	**28**(5)	ca. 27(2)
R. laciniatus	12	**28**(8)	ca. 27(2) ca. 26(2)
R. ulmifolius	15	**14**(10)	ca. 13(3) ca. 12(2)

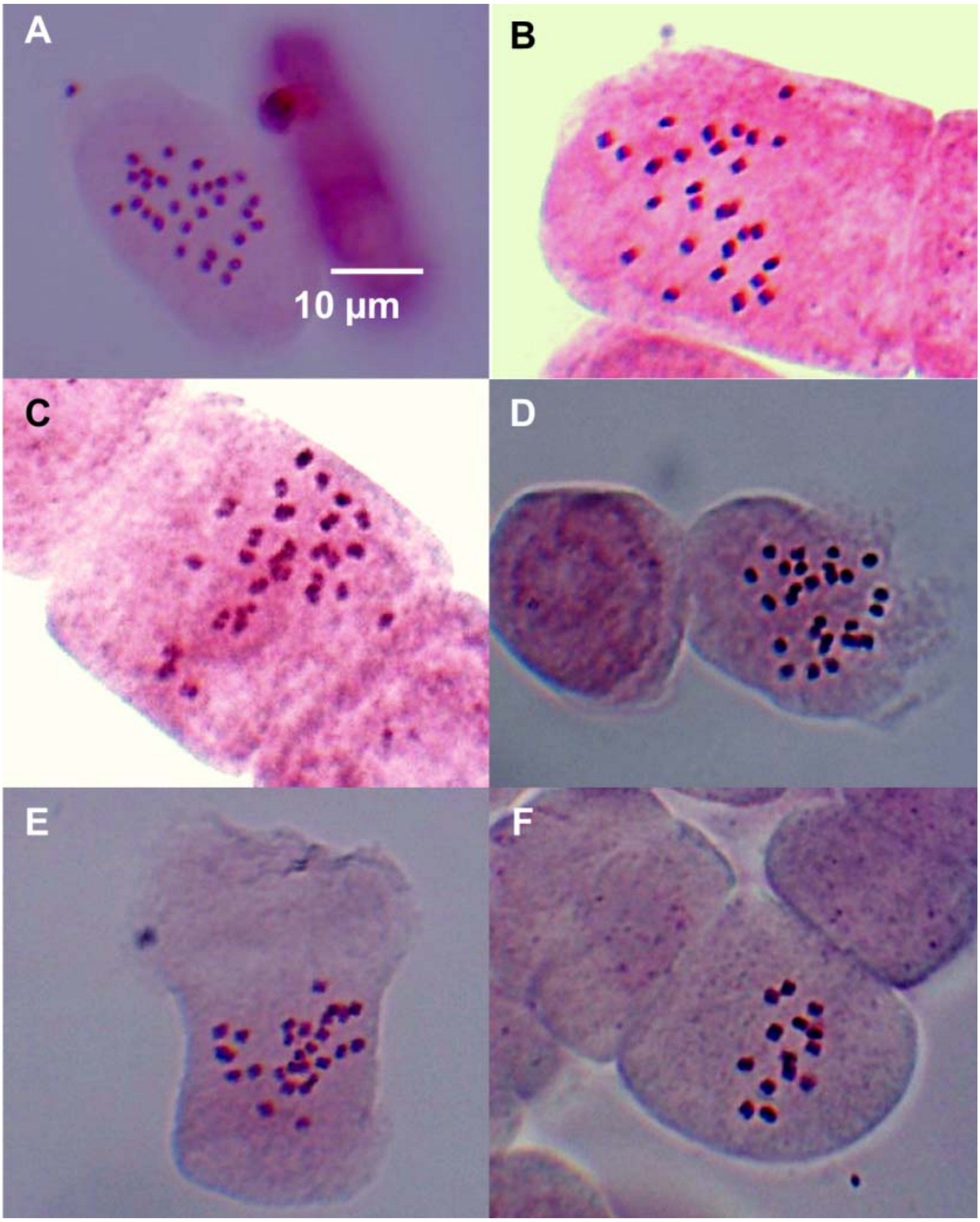

Figure 6.1 Images of chromosome squashes of root tips for six *R. fruticosus* agg. species with chromosomes in metaphase stained with aceto-orcein.
(A) *R. rubritinctus,* (B) *R. anglocandicans,* (C) *R.erythrops,* (D) *R. leucostachys and* (E) *R. laciniatus,* have chromosome numbers of 28 and all are tetraploids. The diploid (F) *R. ulmifolius* has a chromosome number of 14

Flow cytometry findings

Estimates of nuclear genomic size for three putative tetraploid taxas, *R. anglocandicans, R. leucostachys* and *R. erythrops*, were made for some individuals run with internal *Pisum* standards and also for additional individuals, for which an internal standard was not included and in which data on the fluorescence of *Pisum* samples from other runs were used as an 'external' standard as described above. As expected, estimates of DNA content based on external standards were considerably more variable (higher standard deviations) than were estimates derived from runs where internal standards were present (Table 6.2).

Mean 2C values for the three *Rubus* species listed above, with both internal or external standardisation were all greater than 1.34 mean 2C values for these species ranged between 1.34 and 1.68pg with external standardisation and between 1.37 and 1.52pg with internal standardisation (Table 6.2). Samples of the remaining species, *R. rubritinctus, R. laciniatus* and *R. ulmifolius,* were not run together with internal *Pisum* standards and so estimates of genome sizes were standardised externally from data on the geometric means of peaks for *P. sativum* L. calculated from the combined preparations of *P. sativum* L. when run with *R. anglocandicans*, *R. leucostachys* and *R. erythrops*. The mean nuclear genomic sizes of *R. rubritinctus* and *R. laciniatus* estimated in this way were 1.23 and 1.07 pg respectively and that of the putative diploid *R. ulmifolius* was 0.60 pg (Table 6.2).

The peak relationships for *R. anglocandicans, R. leucostachys and R. erythrops* are displayed in Fig 6.1 together with the internal standard, *P. sativum* L., ((9.1pg), Bennett & Leitch, 1995; Doležel & Bartoš, 2005). Relative fluorescence histograms of individual cytotypes of these three *R. fruticosus* agg. taxa against the standard *P. sativum* L. demonstrate the differing relative position of each *Rubus* taxon relative to the standard (Fig 6.3).

Table 6.2 Summary of estimates of mean nuclear genomic size for six taxa of the *R. fruticosus* agg. from Australian plants calculated from the flow cytometric mean relative fluorescence of *P. sativum* L. (2C= 9.1pg). *Rubus anglocandicans*, *R. leucostachys* and *R. erythrops* are were standardised with internal or external samples of *P. sativum* L.

	Nuclear Genomic Size (pg)					
Species	**Internal standard**		**External Standard**		**Number of Samples**	
	2C value	**SD**	**2C value**	**SD**	**Internal**	**External**
R. anglocandicans	1.52	0.07	1.68	0.36	6	19
R. leucostachys	1.47	0.06	1.40	0.26	7	18
R. erythrops	1.37	0.03	1.34	0.35	7	19
R. rubritinctus	-	-	1.23	0.05	-	6
R. laciniatus	-	-	1.07	0.11	-	8
R. ulmifolius	-	-	0.60	0.07	-	17

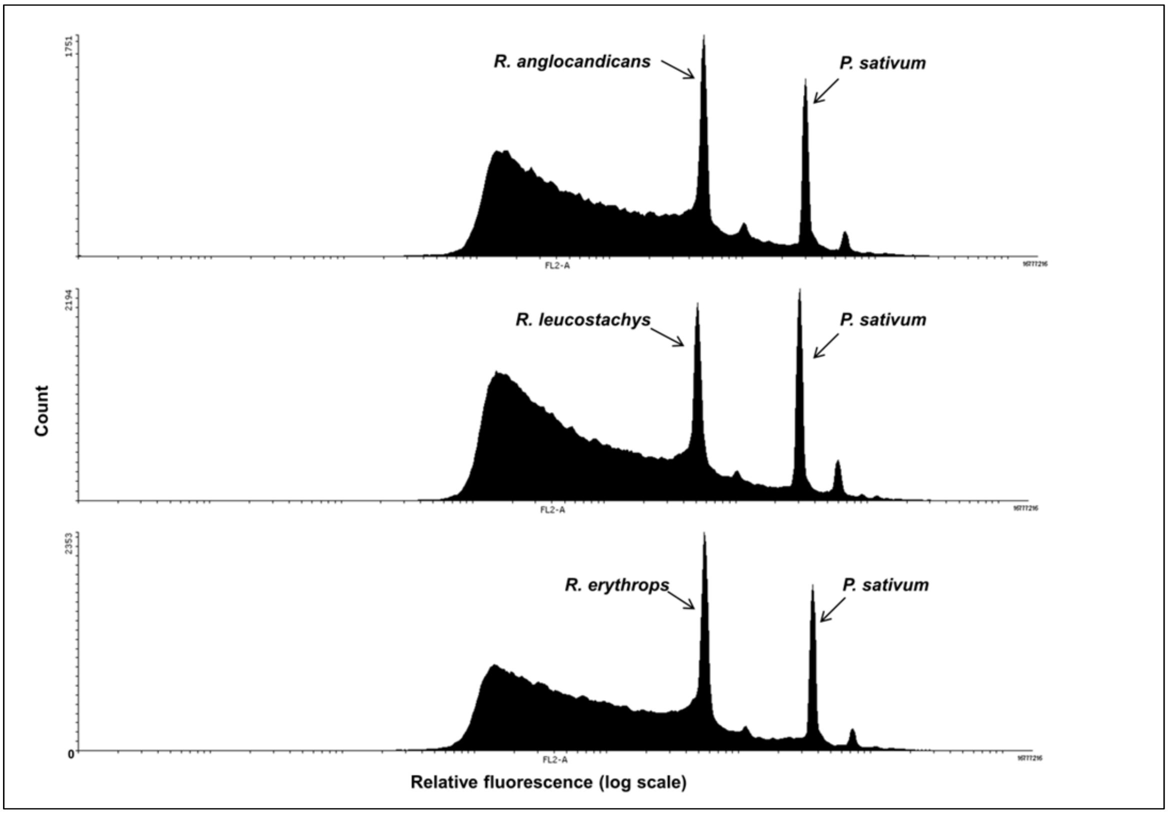

Figure 6.3 Histograms of three cytotypes of *R. fruticosus* agg. taxa and the internal standard *P. sativum* L. The y- axis is the number of nuclei in the 2C peaks of blackberries and the standard (*P. sativum* L.). The relative fluorescence of individual peaks (2C) (log scale) are separated along the x-axis using the forward light scatter channel (FL2-A) with propidium iodine (PI) stain

The putative diploid species *R. ulmifolius (2x)* has the smallest genome size (Table 6.2). The other *R. fruticosus* agg. species examined (all putatively *4x*), *R. anglocandicans, R. rubritinctus, R. laciniatus, R. leucostachys* and *R. erythrops*, have a larger genome sizes, which were calculated relative to the standard *P. sativum* L. (Table 6.2).

Discussion

We surveyed *R. fruticosus* agg. blackberries found in the Mt. Lofty Ranges, South Australia and determined the nuclear DNA size and chromosome numbers for Australian plants of six invasive species in this aggregate. The study provides new evidence on variation in ploidy level and nuclear genomic DNA size of plants in the invasive range in Australia for each of these taxa for some of the introduced blackberries found within the Southern Mt. Lofty Ranges, Flinders Lofty Block, Australia's bioregion IBRA7.

Chromosome Counts

Chromosome numbers are reported in this study for *R. anglocandicans*, *R. leucostachys*, *R. erythrops*, *R. rubritinctus*, and *R. laciniatus* (2n=28) and for *R. ulmifolius* Schott var. *ulmifolius* (2n=14) from their introduced range in Australia and compared with published literature on chromosome counts for *Rubus* reported in Thompson (1997). Chromosome counts reported here for the first five taxa are indicative of tetraploidy, the base number in *Rubus* being seven (Thompson, 1997). The chromosome counts for *R. ulmifolius* Schott var. *ulmifolius* are indicative of diploidy. *Rubus ulmifolius* as the only diploid species of invasive taxa found in Australia is thought to have originated from Britain and our chromosome counts (2n=14) are consistent with this assumption. In the literature, a chromosome number of 2n =14 for *R. ulmifolius* Schott is reported in Heslop-Harrison (1953) and in other studies reported in Thompson's (1997) review, and as a diploid it is considered to readily hybridise with other *Rubus* taxa (Clark *et al.*, 2007).

One of the difficulties in a study such as this is aligning some past species information with data collected more recently. Thompson (1997) noted this issue and attempted to determine currently accepted names for taxa for which counts were recorded using older names that are now taxonomic synonyms of names currently in use. For instance, for *R. anglocandicans* A. Newton a chromosome number of 2n=28 is recorded here from Australian material and differs from the pentaploid chromosome number reported by Thompson (1997) for *R. anglocandicans*, which was originally reported under the taxonomic synonym, *Rubus falcatus* sensu Kalt. from material collected in Northumberland, Great Britain (Heslop-

Harrison, 1953). Thompson (1997) also noted the estimate of chromosome number for *R. anglocandicans* was based on one count and should be confirmed.

A similar issue for *R. rubritinctus* also occurs where a chromosome count of 2n=28 is reported here for Australian material of the species *R. rubritinctus* W. C. R. Watson. Previous estimates of the chromosome number for this species (also 2n =28) were originally reported under the taxonomic synonym *Rubus cryptadenes* Sudre from material collected in Northumberland, Great Britain (Heslop-Harrison, 1953; Thompson, 1997). Our chromosome numbers for *R. erythrops* Edees & A. Newton reported here from Australian material (2n=28) did not differ from that reported by Thompson (1995).

It is worth noting some estimates are from introduced species, such as *R. erythrops* Edees & A. Newton for which chromosome number determinations made by Thompson (1995) appear to be estimated from a collection made at the Riwaka Research Station, Crop Research Division, Motueka, New Zealand. Interestingly, for *R. leucostachys* Schleich. ex Sm., a triploid count (2n =21) and a tetraploid count (2n =28) have previously been reported (Thompson, 1997), including counts (2n = 28) that were made for *R. leucostachys* Sm. from collections in Surrey, Great Britain (Heslop-Harrison, 1953).

Our counts from Australian material of *R. leucostachys* are consistent with the latter counts (2n=28). Our chromosome counts for *R. laciniatus* Willd. (2n=28) from Australian material are consistent with those reported in Thompson (1995) from material collected in Oregon, USA and Australia.

Generally, the information on chromosome numbers reported here for all six species in their introduced range in Australia is consistent with available information for these species from material collected in their native ranges in Europe and Britain (Edees & Newton, 1988) with the exception of *R. anglocandicans* (see above) for which information on ploidy level in its native range needs to be confirmed (Thompson, 1997). There are also unpublished chromosome counts reported for four taxa of the *R. fruticosus* agg. using Australian material by Oliver (1999) who reported counts from shoot tips of circa 28 for DNA phenotypes of *R. polyanthemus* and *R.* affin. *armeniacus* (now *R. anglocandicans* identified in Evans *et al.* (2007) and counts of 28 for *R. leucostachys* and 14 for *R. ulmifolius*. The latter three counts are consistent with results of the survey here.

Nuclear Genomic DNA size

Nuclear genomic DNA size was estimated for six introduced blackberry taxon sampled from the Southern Mt. Lofty Ranges to understand more fully the linkage between genome size and invasiveness in plant taxa. Focussing on our results, *R. ulmifolius* (for which a diploid chromosome number is reported here) eluted at much lower mean 2C values of forward light scatter channel (FL2-A) using PI than the other five *Rubus* taxa, for which tetraploid chromosome numbers are reported here. While the data within the literature are still sparse, our flow cytometry results were generally consistent with the existing literature for other diploid and tetraploid *Rubus* species (Amsellem *et al.*, 2001b; Meng & Finn, 2002; Šarhanová *et al.*, 2012; Krahulcová *et al.*, 2013). For example, the mean nuclear 2C values for diploid species for *Rubus* subgenus *Idaeobatus* and *Rubus* subgenus *Rubus* reported by Meng and Finn (2002) range from 0.47-0.69 and 0.59-0.75 pg respectively. Likewise, Amsellem *et al.* (2001b) found a range of 1.62 pg to 1.82 pg using PI staining and the external standard *Petunia hybrid* in the tetraploid *Rubus alceifolius* Poir. (Rosaceae, *Rubus* subgenus *Malachobatus* Focke) in both its native and introduced ranges. In eastern Europe studies of *Rubus* taxa in their native range demonstrated the following range of genome size variation, 0.75-0.76 pg for diploids, 1.12-1.24 pg for triploids, 1.32-1.64 pg for tetraploids and between 2.22-2.40 pg for hexaploids standardised against several internal and external standards (Šarhanová *et al.*, 2012).

In our study, the three blackberry taxa with tetraploid chromosome numbers and for which internal standards were run, *R. anglocandicans*, *R. leucostachys*, *R. erythrops*, showed mean 2C values ranging between 1.37 and 1.52 pg and between 1.34 and 1.68 pg respectively when using PI staining with *P. sativum* L. as an internal standard or as an external standard. Internal standardisation generated lower standard deviations around the calculated genomic mean when compared with estimates based on external standardisation (Table 6.1), suggesting, as expected, that external standardisation is less reliable although it may still be useful when dealing with difficult material such as members of the *R. fruticosus* agg. For the species *R. ulmifolius* with a diploid chromosome number and using the calibrated external standard *P. sativum* L., 2C was smaller with a mean of 0.60 pg. The findings for these four species with regard to the nuclear genome size are consistent with the established literature for diploid and tetraploid *Rubus* species. For three species, *R. rubritinctus*, *R. laciniatus* and *R. ulmifolius*, only external standards were used here and additional estimations of DNA contents with internal standards would be valuable.

In studies using flow cytometry, the extent of genome size variation across all plant taxa measured is considerable and not well explained (Leitch & Bennett, 2007).

It is interesting to note that Australian plants of six weedy *R. fruticosus* agg. taxa have relatively small genomes (e.g. less than 10 pg), and Bennett *et al.* (1998) reported that weedy angiosperms tended to have smaller mean genome size than the mean for non-weedy taxa they examined. It has been suggested that taxa with small genome size appear less constrained in the range of ecological choices available to them within the habitat and potentially are more adaptable (Knight *et al.*, 2005).

Future considerations

No evidence of hybridisation between diploid and polyploid species of the *R. fruticosus* agg. was detected in the sample of blackberries in the Mt. Lofty Ranges, South Australia examined in this study. Valuable data on the ploidy levels of Australian plants of six introduced species of the *R. fruticosus* agg. have been obtained. Further surveys of cytological variation and of the mode of reproduction of blackberries in this region would also be valuable to examine variation in overlapping populations, particularly where one or more tetraploid species and the diploid species *R. ulmifolius* co-occur. Future monitoring of mixed blackberry populations of different species would enhance our understanding of the extent of variation within this region and of the potential for interspecific hybridisation and the development of new invasive hybrids.

Acknowledgements

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Appendix (App.) Chapter 6

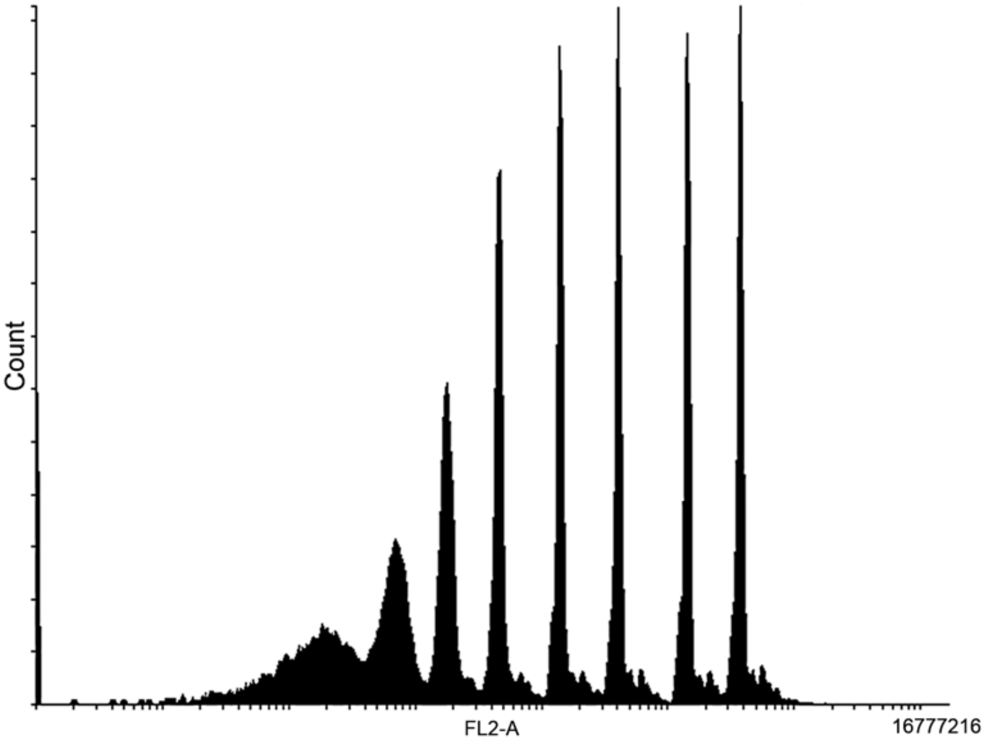

Figure A6.1 Histogram of the relative fluorescence of standard beads (Spherotech® 6 and 8 bead calibration particles) used to calibrate the Accuri C6 Cytometer© . X-scale is particle count against Y-log scale of forward light scatter in channel 2-A (FL2-A) using propidium iodine (PI)

Table A6.1 Summary of *R. fruticosus* agg. taxa used for estimation of chromosome numbers(c) and flow cytometry(f). All material was collected in the Southern Lofty Block, Interim Biogeographic Regionalisation for Australia (IBRA)

Species	**AD voucher**	**Collector**	**Internal 2C value SD**	**External 2C value SD**	**Location**
Rubus anglocandicans A. Newton	AD244147	jm20(c)	-	-	Main Road, W of Nairne
	AD244294	jm47(c)(f, 11)	1.49±0.09	1.90±0.33	Totness Road, near Hahndorf
	AD245550	jm144(c)(f, 4)	1.55	1.36±0.62	Refuse Collection Centre, Hahndorf
	AD244317	jm190(c)	-	-	Swamp Road near Bundaleer Orchard, Lenswood
	AD245470	jm108(c)(f, 5)	1.56	1.54±0.15	Lenswood Oval
	AD245529	jm198(c)(f, 5)	1.55	1.66±0.12	Corner of Piccadilly and Swamp Roads
Rubus erythrops Edees & A. Newton	AD245414	jm231(f, 7)	1.34±0.02	1.14±0.05	Lobethal to Gumeracha Road, near brickworks
	AD245413	jm229(f, 8)	1.39	1.41±0.24	Lobethal to Gumeracha Road
	AD232702	cjb922(c)(f, 1)	1.39	-	Adelaide Hills. W entrance to Horsnell Gully
	AD245042	jm79(f, 1)	1.38	-	Horsnell Gully,W of cold store
	AD245481	jm100(f, 1)	1.37	-	Collins Rd. and Cornish Rd. Cnr. Ashton. Upper reaches of Deep Creek
	AD245414	jm231(f, 8)	-	1.37±0.45	Lobethal to Gumeracha Road, near brickworks
Rubus ulmifolius Schott var. *ulmifolius*	AD244435	jm114(c)(f, 5)	-	0.57±0.11	Old Mt Lofty Railway Station
	AD245048	jm56(c) (f, 7)	-	0.62±0.04	Eagle On The Hill Mountain Trail Bike Park
	AD244307	jm44(f, 5)	-	0.60±0.02	Brown Hill Creek CP

Table A6.1 cont.

Rubus leucostachys	AD245047	jm150$^{(c)\ (f,\ 4)}$	1.46±0.02	1.31±0.35	Road verge, N outskirt Nairne – Woodside Road
Schleich. Ex Sm.	AD245461	jm113$^{(c)}$	-	-	Old Mt Lofty Railway Station
	AD243960	jm73$^{(c)(f,\ 9)}$	1.44±0.08	1.41±0.15	Cleggett Road, Littlehampton
	AD245404	jm249$^{(c)}$	-	-	Summit Road, Mt. Bonython Telecom tower. Mt. Lofty
	AD245060	jm142$^{(c)(f,\ 12)}$	1.50±0.04	1.42±0.32	Littlehampton, W end of town Kookaburra Lane by bridge
Rubus rubritinctus	AD245472	jm107$^{(c)(f,\ 1)}$	-	1.21	Lenswood Oval
W.C.R. Watson	AD244417	jm90$^{(c)(f,\ 3)}$	-	1.25±0.05	Reserve Lenswood Rd
	AD245454	jm83$^{(c)(f,\ 2)}$	-	1.22±0.08	Lenswood Memorial Park, Swamp Road
Rubus laciniatus	AD245173	jm97$^{(c)(f,\ 8)}$	-	1.07±0.11	Lenswood
Willd.	AD232704	cjb923$^{(c)}$	-	-	Adelaide Hills. W entrance to Horsnell Gully
	AD232713	cjb944$^{(c)}$	-	-	Adelaide Hills. Lenswood. Behind General Store

Note: collector # code; jm = John Marshall (Flinders University) ; cjb = Christopher Brodie (State Herbarium SA)
Superscripts after the collector # (c) indicates the plants from which root tips were collected for use in the survey of chromosome numbers and (f, *n*) indicate the number of flow cytometry samples
Pisum sativum L. was used for internal and external standardisation and calculation of the 2C values of nuclear genomic size in picograms (pg)

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Chapter 7

General discussion

This thesis investigated the biology and ecology of individual species of blackberries that have become major weeds in Australia.

In this final section of the thesis, I shall summarise and discuss the major findings of the research chapters and suggest ways to develop the research of invasive blackberries into the future.

The chapters in the thesis are linked by a general theme of seeking to understand more completely the ecological and biological preferences of individual blackberry taxa that influence or enhance invasion in Australian plant communities. Ultimately, the knowledge of the preferences of individual species presented here gives a better estimation of which species are of the greatest threat to areas of high biodiversity value in Australia.

In the initial part of my thesis, I presented some key general hypotheses, which were used to form the research questions in each research chapter.

In my research, I used an approach integrating species distribution modelling and environmental niche modelling. I also employed biological and ecological experiments (both in the field and glasshouse environments) to define and understand the individual preferences and competitive behaviour of selected blackberry species within their respective niches.

I investigated the ploidy level and nuclear genome size of a limited number of individual blackberry taxa from material collected in the Mt. Lofty Ranges, where the pattern of distribution of cytotypes of *Rubus fruticosus* L. aggregate species has been poorly investigated and is unknown for most species.

Understanding the ecological differences between closely related blackberry species is likely to enhance existing control and management systems and provide better outcomes where biodiversity conservation is the prime concern.

General findings

The general findings of my research show that the species of European blackberries (*R. fruticosus* agg.) in Australia display differing niche preferences. They also have different population ranges and niches (some overlapping) within the Mt. Lofty Ranges and within Australia that are influenced by a combination of ecological and biological preferences and the biological attributes of each species.

Results from all chapters except chapter 6 provided evidence to suggest that *R. anglocandicans* is the dominant invader in Australian natural systems and is more adapted to ecological conditions in Australia than the other blackberry species studied.

Findings chapters 2 & 3-Species distributions and niche modelling

The first two research chapters overlap and expand the knowledge of the potential range of each blackberry species studied and quantify the similarity and differences of the niches between the individual species of blackberries. Evidence in Chapter 2 indicates a potential for these species to expand their ranges into much of southern Australia, particularly at the margins of the present distributions of some species and in greater regional areas such as Western Australia. Western Australia has only two invasive species of the fifteen known to occur in Australia but appears to be at risk of invasion by most of the species examined in this study.

The predictions of the SDM models showed the potential environmental niches of almost all species exceed the geographical presence currently indicated by herbarium records for each species, implying most species have not reached the limits of their introduced ranges.

In Chapter 3, a detailed assessment of the difference between the climatic niches of blackberry species indicated the niches of the closely related blackberry species were similar but sometimes significantly differentiated. The climatic niches were most similar between *R. leucostachys* and *R. anglocandicans* and most dissimilar between *R. leucostachys* and *R. erythrops.* Interestingly, *R. leucostachys* occupies a similar geographic area as *R. anglocandicans* (based on the records in the State Herbarium AD), but is found at much lower densities within South Australia, which indicates that herbarium collections can be less reliable in reflecting the biotic and abiotic elements of species niches, which are used in analyses of this type. I suggest from these results that some species that currently occupy similar geographic areas may occupy differing climatic niches.

A consistent result from Chapters 2 & 3 is that *R. leucostachys* and *R. anglocandicans* are the most common species found and the most likely to extend their ranges over extensive areas of southern Australia. By contrast, the other species studied except for *R. ulmifolius* are more restricted in their bio-climatic niches and appear less likely to be invasive than *R. leucostachys* and *R. anglocandicans. Rubus ulmifolius* appears to be an adaptable species based upon the extent of its potential range predicted by SDM although it is characterised by a narrow climatic niche potentially influenced by topography that overlaps with those of other species. This finding is noteworthy, as a diploid species such as *R. ulmifolius* may be a progenitor species important in the process of hybridisation as discussed in Chapter 6.

Findings chapter 4-Testing invasion of native vegetation communities in the Mt. Lofty Ranges

Results from my study of native vegetation in South Australia show that of the ten or so blackberry species that occur in the region only five species are strongly associated with native vegetation. *Rubus anglocandicans*, *R. erythrops* and *R. rubritinctus* are the most common invaders of native vegetation communities with *R. leucostachys* and *R. laciniatus* occurring at very low frequencies in the field survey of native vegetation. The most common species, *R. anglocandicans,* is more tolerant to broad climatic extremes compared with the other species. Both *R. erythrops* and *R. laciniatus* are more restricted in their niches and are positively associated with high soil moisture levels. One of the least frequently encountered species in native vegetation, *R. rubritinctus,* prefers watercourses in low gradient open valleys typical of the eastern Mt. Lofty Ranges. One of the most interesting findings was the lack of plants of *R. leucostachys* in the native vegetation survey plots. *Rubus leucostachys* is the second most common species found in herbarium records (AD) and occupies a current range that is similar to that of *R. anglocandicans*. The marked difference though between the field survey results and herbarium data appears predominantly due to the preference of *R. leucostachys* for more open areas away from dense native vegetation. *Rubus leucostachys* may overlap geographically with *R. anglocandicans* as demonstrated by my SDM but it is significantly different in its bioclimatic niche in the landscape. Contrasting closely related blackberry species by their ecology and biology and their association with native vegetation demonstrates that individual species have different capacities to invade native vegetation.

One restriction on the interpretation of the models from my survey is that modelling of some species was based upon relatively small data sets, which could be resolved by more intensive sampling within the region.

Findings chapter 5-Competition of blackberries with native plants

Competition between selected blackberry species and native species was tested using paired plantings under shade-house and field conditions. The success of introduced blackberries in Australia suggests that they are likely colonisers of native vegetation communities but few studies have examined the extent of variation in both habitat preferences and invasiveness between differing blackberry taxa.

The general hypothesis that invasive species are better competitors assumes invasive taxa possess a suite of life history traits that enable them to compete more effectively in new ranges. This broad-based view includes complex plant-plant interactions of competition that form and influence ecosystem function and appear to play an important part in the success or failure of new invaders in new ranges (Navas & Violle, 2009). The functional morphology of invasive *Rubus* species in the western United States was found to be a factor enhancing their competitive ability over native *Rubus* species and appears to link life history traits to invasion success (Caplan & Yeakley, 2006, 2010, 2013).

In my study, I focussed on examining the competitive ability of three different blackberry species to try to understand how plant-plant interactions between exotic blackberries and two native species may influence the success or failure of an invader. I found *Acacia provincialis* was not significantly reduced in its growth but the above and below ground vigour of *Leptospernum continentale* was significantly reduced. Of the three blackberry treatments, *R. anglocandicans* had the greatest effect in reducing the growth of *L. continentale*, and *R. leucostachys*, the least. The findings in this study demonstrate a significant difference in the competitive ability of a few blackberry species over selected native taxa. Further work is required to test the hypothesis as to whether nitrogen-fixing taxa (such as *A. provincialis*), or communities rich in nitrogen-fixing taxa, are relatively more resilient to invasion by *Rubus* and other invasive taxa.

The results from the field experiments were inconclusive as many plants were lost through herbivory.

Findings chapter 6-DNA content and chromosome counts

In this chapter, I assessed the ploidy of six blackberry species found in the Mt. Lofty Ranges region. The mode of reproduction (assumed to be facultative apomixis) of the introduced species of blackberries which are considered likely to be highly clonal is thought to be the same as in their native ranges (Evans *et al*., 2007). Generally, estimates of chromosome numbers from plants in their invasive ranges can help to determine the breeding systems of individual species and whether invasion has been associated with changes in ploidy. My estimated chromosome numbers for the species *R. anglocandicans* A. Newton, *R. rubritinctus*, *R. erythrops* Edees & A. Newton, *R. leucostachys* Schleich. ex Sm. and *R. laciniatus* Willd. are 2n=28, or ca. 28, which is indicative of tetraploidy. These estimates for selected individuals collected within the region are consistent with available information from the taxa collected in their native ranges in Europe and Britain and from some introduced ranges (Thompson, 1997). We also recorded the chromosome number of the putative diploid *R. ulmifolius* var. *ulmifolius* as 2n=14.

My findings using cytological techniques are important in comparing ploidy levels of the blackberries found in the Mt. Lofty Ranges with records from their native ranges. As this survey included only a few individuals of five species of the ten or so species present in the Mt. Lofty Ranges, further surveys of more taxa, both within this region and in Australia more broadly would be required to detect the presence of any hybridity that may exist in populations. While studies of blackberry populations in Europe have detected the presence of triploid cytotypes as indicative of the occurrence of sexual reproduction and hybridisation (Šarhanová *et al.*, 2012), my results do not provide any evidence that hybridisation has occurred between populations within the Mt. Lofty Ranges.

Small genome size has been linked to invasiveness of some plant species (te Beest *et al.*, 2012). Smaller genome size as a life history trait appears to influence the invasive fitness and ecological adaptability of taxa in introduced ranges (Bennett, 1972; Bennett *et al.*, 1998; Vinogradov, 2003; Varela-Álvarez *et al.*, 2012). In my study, small genome sizes of less than 10 picograms were determined for six individual *Rubus* taxa found within the Mt. Lofty Ranges using flow cytometry techniques.

Future Directions and Recommendations

Are some Rubus taxa more invasive than others and are some areas more vulnerable to invasion?

In Australia some taxa of the *R. fruticosus* agg. appear to be more invasive based upon their frequency of occurrence in the Australian landscape. Our study provides some empirical evidence that may help to explain this variation.

In our spatial modelling of *Rubus* taxa in Chapter 2, we found significant variability among species with respect to their potential invasive ranges and suggested that *R. anglocandicans* and *R. leucostachys* may expand their ranges more to occupy larger ranges in Australia when compared with *R. erythrops*, *R. rubritinctus* and *R. laciniatus*. Species distribution modelling as a static representation of a species potential niche does not take into account directly the effects of competition but the evidence in our competition study, for the three blackberry species included in the study, showed a pattern of effects which was consistent with the findings in Chapter 2. In Chapter 3, *R. anglocandicans* also appeared as the most differentiated taxon in the niche similarity test between seven paired species of invasive *R. fruticosus* agg. taxa. We found the niches calculated for the combination of *R. leucostachys* vs. *R. anglocandicans* were not identical against the overlap scores calculated from actual occurrence data while the niches of *R. erythrops*, *R. rubritinctus* and *R. laciniatus* against *R. leucostachys* were more different. With regard to the invasion of native plant communities in particular, in the field survey of the presence and absence of *R. fruticosus* agg. taxa in native vegetation in the Mt. Lofty Ranges in Chapter 4, we found five species to have invaded native vegetation communities. We found *R. anglocandicans* was the most frequently encountered in the survey with *R. erythrops* and *R. rubritinctus* less frequently encountered, but all three were common invaders of native vegetation communities. The occurrence of these three species showed significant but differing correlations with aridity, soil moisture, sunlight intensity and the presence of bare ground. A model of the bio-climatic preferences of *R. leucostachys* was not established due to its absence in quadrats in the survey of native vegetation but it is likely to be an important invader at the margins as it has a modelled potential (Chapter 2) to occupy larger areas across southern Australia.

Rubus anglocandicans based on our results appears to be the most invasive species of the *R. fruticosus* agg. in Australia. Further studies to test the ecological and biological preferences of individual species over broader areas of Australia supported by precise and accurate spatial data with concurrent expansion of collections of new vouchers are required to not only improve the collection sample size for some taxa but also to increase the likelihood of detection of hybridisation within overlapping populations. Hybridisation between species is more likely in most southern states of Australia where more species occur.

Rubus ulmifolius is of particular concern in this regard being the only diploid species and one that is found over broad areas, and it would likely be a progenitor species in hybridisation events with other taxa.

We recommend further research should be undertaken and this includes, but is not limited to, the expansion of collections of material of every exotic blackberry present within Australia including both vouchered material with locality data and material for DNA analysis. This routinely includes developing a greater understanding of the locations of individuals and populations of each species, in both Australia and their home ranges. An expansion of the understanding of the niche traits and competitive behaviours of individual species with further studies and experiments, both in the field and the laboratory, will help to unravel further the biological and ecological characteristics of each of the blackberry species in Australia. Ultimately, research targeting invasive species should add to the knowledge base for adaptive management systems and while this thesis has provided new information regarding the biological and ecological preferences that characterise *R. fruticosus* agg. taxa in their introduced ranges within Australia, further work is required particularly in the Australian context.

Species distribution modelling of invasive blackberry species is a valuable tool in understanding a population's potential extent although SDM is only as good as the robustness of the datasets for the target species involved. Further expanding collections of blackberry specimens with accurate locality data is of great importance as it more broadly samples the environment for the bio-climatic variable data used in modelling. The collection of further home range data from across Great Britain and Europe also would add power to the SDM of all species of blackberry found within Australia. Niche traits of individual species in particular are of great interest as they can be measured and compared between invasive species to enable researchers and land managers to identify not only ecological differences among individual species in an aggregate such as *R. fruticosus* but also to adapt management practices in accordance with these traits. Adaptive management of blackberries within Australia is an ongoing activity and the likelihood of successful removal of individual species locally and regionally will be greater if adaptive management incorporates a species-by-species approach based upon the individual biological and ecological traits of each species. Land managers within the Mount Lofty Ranges (MLR) and within the regions of South Australia (Yorke Peninsula and the state's South East) should be more aware of the invasiveness (detected in our study) of *R. anglocandicans* in particular and undertake searches based upon our SDM findings for the presence of this and other blackberry species. Regional land managers should also be aware of the potential of the spread of most other species of blackberries that are currently concentrated in the MLR.

Direct removal of blackberries should be undertaken where multiple species occur in the MLR and field programs should remove large colonies (particularly those of *R. anglocandicans*) in preference to smaller outlier colonies to further limit the primary spread by seed distributed by avian species, foxes and native herbivores.

Currently, the numbers of exotic species of blackberries in the southern states of Australia are not the same for each state. This may indicate a different history of introduction. We recommend, based on our evidence, a program to increase public awareness nationally of the need to limit further spread of species into neighbouring states, particularly in Victoria and Western Australia.

Competitive interactions between blackberries and native plant taxa

Invasion by exotic species provides an opportunity to not only study the threats they represent to habitats and ecological systems but also to learn more about the dynamics of competition within communities and at the individual plant level (Crooks, 2002).

Competitive interactions among individual plants appear to play a major part in the success or failure of invading species in new ranges although it is likely that a different combination of competitive mechanisms applies to each invasion scenario (Yelenik *et al.*, 2004; Richardson, 2011; French *et al.*, 2014; Gioria & Osborne, 2014; H.H.T. Prins & I.J. Gordon, 2014).(Callaway & Aschehoug, 2000; Stinson *et al.*, 2006). Australian plants such as some *Acacia* spp. that have invaded new habitats are understood to directly modify their new ecosystem by altering system level flows of nutrients food and physical resources thereby outcompeting native vegetation (Yelenik *et al.*, 2004; Roura-Pascual *et al.*, 2009). Known as ecosystem engineers, these species may provide some defence in their native ranges against invading exotics.

Exotic blackberry species as successful global invaders have been shown to demonstrate superior fitness in some studies over native species (Randell *et al.*, 2004; Yeakley & Caplan, 2008; Caplan & Yeakley, 2013).

The limited evidence from my study suggests some native species are more capable of resisting invasion from blackberries than others. Further paired studies, both under controlled environmental conditions and in the field, are necessary to help identify the traits and mechanisms that may enable native plants to compete more effectively with invasive blackberries. The value of understanding competition in pair-wise trials in both field and garden studies is that such studies will help to identify Australian native plants that may be used in conservation and restoration. Further monitoring invasion fronts may also help to identify native plant species that are capable of resisting invasion by blackberries.

The importance of understanding the reproductive behaviour of alien plants in invaded ranges

The reproductive behaviour of alien plants has been noted as a primary mechanism to aid and enhance of invasion in new ranges (Richardson & Pyšek, 2006; Barrett, 2011). The example of the notable global invader, *Spartina* (Poaceae), shows how through the processes of hybridisation and polyploidy new hybrids may arise and occupy new niches (Ainouche *et al.*, 2009; Castillo *et al.*, 2010).

In South Australia the presence of multiple species of the *R. fruticosus* L. agg. (including a potential diploid progenitor *R. ulmifolius*) poses a potential threat to the region through similar processes. Understanding of the reproductive behaviour of the blackberry species found in the Mt. Lofty Ranges is still incomplete and needs to be more fully investigated with detailed analysis of the hybridisation status of mixed populations of blackberries in the Mt. Lofty Ranges. Given the identified variability in invasiveness among blackberry species as shown by this study of the *R. fruticosus* L. agg., monitoring of the hybridisation outcomes, if any, in field populations would be wise.

My study of blackberries is unusual in that I used a detailed spatial modelling approach married with biological and ecological experiments to investigate the ecological differences in invasiveness between a group of closely related species. Such a multidisciplinary approach appears to be the path of general concensus towards developing a more general understanding of how exotic species integrate into new ecosystems and also yielding more information for options for biodiversity management (Richardson, 2011).

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