Determining the invasive status of Australian *Acacia* species in South Africa, and the potential for eradicating species with limited distributions

Nkoliso Magona

Thesis presented in partial fulfilment of the requirements for the degree of Master of Science in Botany at Stellenbosch University (Department of Botany and Zoology)

Principal Supervisor: Prof. John R. Wilson

Co-supervisor: Prof. David M. Richardson

Department of Botany & Zoology Faculty

of Science Stellenbosch University

March 2018

DECLARATION

i. By submitting this thesis/dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.
Date: March 2018

ii. Chapter 3 was initiated as part of an uncompleted Master's thesis in 2012 (George Sekonya), and ~ 10 % of the data used in Chapter 3 was gathered in this work. All other data collection and analyses are my own original work unless otherwise acknowledged. Details on contributions to the thesis are provided at the start of each specific chapter. This thesis contains a single bibliography to minimise duplication of referencing across the chapters.

Abstract

While widespread invasions of Australian acacia species (wattles) have been fairly well documented, very little is known about species that have no substantial commercial value or those that are not well-established invaders yet. South Africa has the highest number of invasive wattle species in the world. These have had negative impacts on the environment and socio-economy. However, the last detailed inventory of the group in South Africa was based on data collated forty years ago. In addition, there are several species with small naturalised populations that might pose a future risk. A recent study quantified different aspects of this "invasion debt" for wattles, both for South Africa and globally and found out that southern Africa has a large invasion debt. In Chapter 2 I aimed to determine how many Australian *Acacia* species are known to have been introduced to South Africa, which species are still present and what their status is. I visited herbaria, arboreta, botanical gardens and conducted field surveys in order to compile a list of introduced wattles, and used DNA bar-coding to confirm the identity of these species. I found records for 114 wattle species introduced into South Africa, but I found the presence of only 50 species. Seventeen of these species are invasive (16 are in category E, one in category D2 in the Unified Framework for Biological Invasions); eight species have naturalised (category C3); and 25 species are present but are not known to produce seed in South Africa (category C1). Four of these occur in the Western Cape (three on the Cape Peninsula, *A. piligera, A. retinodes* and *A. viscidula*; 1 near Paarl, *A. adunca*) and two species, *A. cultriformis, A. fimbriata* in Grahamstown in the Eastern Cape. In Chapter 3, I focus on the potential to eradicate these six naturalised wattle species from South Africa. I carried out a systematic survey of populations and the surrounding areas. For each plant, I recorded plant canopy, height, stem basal diameter, presence or absence of reproductive structures and GPS coordinates. I then cut or pulled out the plants. I assessed the risk posed by these species using Australian weed risk protocol and lastly, I determined the current size of the seedbank for these species. Risk assessment showed that all of these species have high potential impact, hence, they should be considered as a threat. All of these species except *A. retinodes* can reach reproductive maturity within a year and three of these species have large seedbanks. If control efforts can continue to prevent reproduction, eradiation will be a matter of reducing the seed banks across the limited distributions for these species. I conclude that eradicating five of the species is feasible and annual clearing resurveys are recommended in order to prevent production of seeds. *Acacia cultriformis* was clearly at some point used in the ornamental plant trade and there are many isolated populations. This makes it difficult to find all plants and eradication is unfeasible. I conclude with Chapter 4, where I provided recommendations for listing and management.

Keywords: Australian acacias, biological invasions, eradication, introduction status, invasive species, management plan, tree invasions.

Opsomming

Terwyl wydverspreide indringing van Australiese akasia-spesies (wattels) redelik goed gedokumenteer is, is baie min bekend oor spesies wat geen beduidende kommersiële waarde het nie of die wat nog nie gevestigde indringers is nie. 'n Onlangse studie het verskillende aspekte van die "indringingskuld" vir wattels gekwantifiseer, beide vir Suid-Afrika en wêreldwyd, en het uitgevind dat Suider-Afrika 'n groot indringingskuld het, selfs vir wattels wat nog nie wydverspreid is nie. Dit beteken dat daar 'n beduidende toename in die algehele ekologiese en ekonomiese impakte van wattels sal wees.

Suid-Afrika het die grootste aantal indringer wattel spesies in die wêreld, en dit het negatiewe impakte op die omgewing en sosio-ekonomie. Tog was die laaste gedetailleerde inventaris van die groep in Suid-Afrika gebaseer op data wat veertig jaar gelede ingesamel is. Daarbenewens is daar verskeie spesies met klein genaturaliseerde bevolkings wat waarskynlik 'n toekomstige risiko kan veroorsaak. Met hierdie studie het ek gepoog om vas te stel: hoeveel Australiese *Acacia* spesies is ingebring na Suid-Afrika, watter spesies is nog steeds teenwoordig en wat hul status is (Hoofstuk 2). Ek het herbaria, arboreta en botaniese tuine besoek, ook is veldopnames gedoen om 'n lys van ingevoerde wattels saam te stel. DNA-kodering is gebruik om die identiteit van hierdie spesies te bevestig. Ek het rekords gevind vir 114 wattle spesies wat in Suid-Afrika ingebring is, maar ek kon slegs 50 spesies steeds vind. Sewentien van hierdie spesies is indringers (16 is in kategorie E, een in kategorie D2 in die ''Unified Frame Work for Biological Invasions''); 8 spesies is genaturaliseer (kategorie C3); en 25 spesies is teenwoordig, maar is nog nie waargeneem om saad in Suid-Afrika te produseer nie (kategorie C1).

Ek het op ses genaturaliseerde wattel spesies uit vorige populasie opnames gedoen. Hiervan het 4 spesies in die Wes-Kaap voorgekom (3 Kaapse skiereiland: *A. piligera*, *A. retinodes* en *A. viscidula*; 1 naby Paarl: *A. adunca*) en twee spesies kom voor in die Oos-Kaap (Grahamstad: *A. cultriformis* en *A. fimbriata*). In Hoofstuk 3 fokus ek op die moontlikheid om genaturaliseerde wattel spesies uit te wis in Suid-Afrika. Ek het 'n

sistematiese opname gedoen oor bevolkings en hul omliggende gebiede. Vir elke plant het ek die volgende aangeteken; kroon deursnee, hoogte, basale stam deursnee, teenwoordigheid of afwesigheid van reproduktiewe strukture en GPS koördinate. Dan trek ek die plant uit of kap dit af. Deur die Australiese onkruidrisiko-protokol te gebruik, is die risiko van hierdie spesies geassesseer en laastens is die huidige saadbank grootte per spesie bepaal. Risikobepaling het getoon dat al hierdie spesies 'n hoë potensiële risiko-impak het, daarom moet hulle as 'n bedreiging beskou word. Al hierdie spesies kan reproduktiewe volwassenheid bereik binne 'n jaar en drie van hierdie spesies produseer ' groot hoeveelhe saad.

In Hoofstuk 4 het ek aanbeveel dat hierdie wattels gelys moet word en bestuurstrategieë word verskaf. Aangesien daar nie meer volwasse plante is nie, net hul saadbank, en beperkte lokale verspreidings, het ons tot die gevolgtrekking gekom dat die uitroeiing van hierdie spesies uitvoerbaar is, en dat jaarlikse opvolg her-opnames aanbeveel word vir die voorkoming van nuwe saadproduksie.

Sleutelwoorde: Australiese akasias, biologiese indringings, uitwissing, indringerspesies, bestuursplan, boom indringers, indringer status.

Acknowledgements

I thank my supervisors, John Wilson and David Richardson for their guidance and support during this project.

I acknowledge the funding received from the Department of Environmental Affairs through its funding of the Invasive Species Programme (SANBI), the DST-NRF Centre of Excellence for Invasion Biology and the Department of Botany and Zoology at Stellenbosch University for their support.

I thank all South African botanical gardens, herbarium staff, and administrative staff for their generous assistance.

The administrative staff at the Centre for Invasion Biology (Christy, Karla, Mathilda, Rhoda and Erika) for their help over the years.

Special thanks to my family and friends for their key role in supporting me during this study.

CONTENTS

List of figures

List of tables

Chapter: 1 General introduction

The introduction of alien species to many countries has brought many socio-economic benefits in the form of timber, fuel wood, tannin and other products (Kull et al. 2011). However, many of the species have the potential to become invasive (Rouget et al. 2016). Hence, information about the whereabouts of these species is essential in order to keep track the movement of these species (Wilson et al. 2011). Data about biodiversity from historical records hold a great value in keeping the distribution range of species known and as reference material. Alien species lists give an indication of the species that are already present and their current invasion status and help to inform policy makers (McGeoch et al. 2012; Regan et al. 2002). However, there are a number of errors and biases that typically exist in such species lists: insufficient survey information, inappropriate data resolution, undocumented data, inaccessible data, lack of sufficient information on indigenous distribution range, incomplete information, misidentification and un-described species, misidentification and synonyms (McGeoch et al. 2012; Regan et al. 2002). There is a need to search for the sources of these errors and biases in the published literature, and in museums and herbaria to create more comprehensive, accurate and reliable databases.

Australian acacias are a good study group to address the problems associated with listing of alien species for several reasons: (1) introductions and plantings of species in this group have been fairly well documented; and (2) Australian acacias are amongst the most widely transferred species and well-studied invasive plant species around the world.

Australian acacias have been used to serve a wide range of different needs (Le Maitre et al. 2002; Kull and Tassin 2012). For an example, the introduction of Australian acacias played a major role in improving livelihood of communities; (Kull et al. 2011; van Wilgen et al. 2011), and economic growth (Gaertner et al. 2009; Griffin et al. 2011; Richardson et al. 2011; Richardson and Rejmánek 2011; Moore et al. 2011). However, some species of Australian acacias are highly invasive and pose a threat to biodiversity by transforming ecosystems (Le Maitre et al. 2000, 2011; Richardson and Van Wilgen 2011). This has created a conflict of interest between people managing natural resources and those who benefit from acacias in various ways (Carruthers et al. 2011; van Wilgen et al. 2011; van Wilgen and Richardson 2014).

There are ~1022 *Acacia* species (formerly grouped in the subgenus *Phyllodineae*), of which 386 species are known to have been moved by humans to areas outside their native ranges; at least 71 have become naturalized, and at least 23 have become invasive (i.e. have spread over substantial distances from planting sites) (Richardson et al. 2011). Knowledge of the introductory history of these species is crucial in order to understand and predict their performance (Wilson et al. 2011; 2014; Motloung et al. 2014; Panetta et al. 2011). However, the extent and the patterns of those species are poorly known and this could result in a high invasion debt (Rouget et al. 2016). The realisation of the invasion debt could lead to more widespread invasions in the future and greater impacts.

There is a large body of literature on many aspects of Australian acacias from the cellular level to how they behave in their introduced range (Le Roux et al. 2011; Richardson et al., 2011; Wilson et al. 2011). The long introductory history and widespread transfers of Australian acacias into novel ecosystems around the world has resulted in an opportunity to investigate factors that drive the success and failure of introductions, and how native species respond to such events (Richardson et al. 2011). As a result, there is a growing body of research on the impacts associated with naturalized and invasive populations of acacias (Ross 1975; Le Maître et al. 2011; Richardson and Van Wilgen 2011). If the invasion debt were realised, there could be a substantial escalation in the overall ecological and economic impacts of Australian acacias (Richardson et al. 2015). One way of reducing this invasion debt is through eradication of those species that are still at an early stage of invasion and for which total removal is still feasible.

South Africa has a long history of introductions and invasions of Australian acacias (wattles). Wattles were first introduced to South Africa by the Cape Colonial Secretary in the early 18th century to bind sand dunes on the Cape Flats, a low-lying area southeast of Cape Town (Ross 1975; Poynton 2009), in particular *Acacia cyclops, A. longifolia* and *A. saligna*. Later, species of commercial value, including *A. decurrens*, *A. mearnsii* and *A. melanoxylon*, were introduced for timber (van Wilgen et al. 2011).

According to Carruthers et al. (2011), the introduction of Australian *Acacia* species into the country was criticised by certain organs of state and by some sectors of society as they saw the planting of these trees as unnecessary and expensive. In contrast, Kull et al. (2011) reported that the planting of alien trees created job opportunities for poor rural people. Most of the plantings were done by the Forestry Department. Poynton (2009) reported that during the 19th century government schemes were implemented to promote the widespread planting of acacias as it provided employment for many people. Repeated forestry trials were done in different stations across the country and most of these places were left unmanaged (Poynton, 2009).

Prior to this study, sixteen Australian *Acacia* species were considered invasive in South Africa (Wilson et al. 2011). Another four species were known to have naturalized, and another two to be reproducing locally and are probably best categorized as “casual aliens” (definitions of invasive, naturalized and casual species follow Richardson et al. 2000). No other region of the world has received as many introductions of Australian *Acacia* species or has as many invasive species (Richardson et al. 2011; Richardson and Rejmánek 2011).

1.1. Eradication

Eradication is the elimination of every single individual of a species from an area to which recolonization is unlikely to occur (Myers et al. 1998). This is often set as a management goal that, if achieved, will reduce future potential negative ecological and socio-economic impacts (Gheradi and Angiolini 2009; Panetta 2007; Mack and Lonsdale 2002). However, eradication of plant species can be time consuming and expensive (Rejmánek and Pitcairn 2002; Panetta, 2007; Wilson et al. 2017). Eradication is sometimes not an appropriate goal for management, and many resources have been wasted on chasing eradication in situations where eradication was never particularly feasible (Simberloff, 2009).

For eradication projects to be successful, the targeted species must be well-studied and the project must be started before the species becomes widespread (Wilson et al. 2017; Panetta 2007; Simberloff 2003). Adequate resources need to be ensured before the project starts to allow for post-removal surveys, and during control there should be regular follow-ups (Simberloff 2009). There are two stages of weed eradication: (1) the active phase which involves the control of established plants and new recruits; and (2) the monitoring phase where no plants have been found after the control phase, but there is still a possibility of the plants being present due to the existence of the soil seed banks or if individual plants have been missed.

1.2. Thesis outline

There are two main aspects to this project. First (Chapter 2), the study was set out to assess the status of *Acacia* species in South Africa. The study categorised invasion status of populations according to the stages of the introduced-naturalized-invasion continuum defined by the unified framework for biological invasion by Blackburn et al. (2011). Second, it assessed the feasibility of eradicating some of these species.

The plantings and introductions of Australian acacias as exotics are fairly well documented (see Poynton 2009). However, the study by Poynton (2009) focussed primarily on forestry introductions and is seriously out of date as most of the work was done in the 1970s. For example, species such as *A. stricta* were not mentioned (Kaplan et al. 2012) and the results of recent surveys are not included (e.g. Zenni et al. 2009’s study on *Acacia paradoxa*).

Chapter 3 of this study focussed on the management of naturalized *Acacia* species in South Africa and assessed the feasibility of eradicating them. Wilson et al. (2014) indicated that to manage biological invasions effectively, data on the distribution and current status of invasive alien plants is very important together with potential range size (estimated using species distribution models). A recently study by Motloung et al. (2014) used species distribution models to assess potential range of less widespread species. Motloung et al. (2014) did some preliminary surveys in Pretoria on recorded ornamental acacia species (*A. floribunda*, *A. pendula* and *A. retinodes*). Adult plants of *A. pendula* and *A. floribunda* were present, but neither species appeared to have naturalised. Young pods were found on *A. pendula*, but no seeds were observed, Motloung et al. (2014) cautiously classed *A. pendula* as per C2 (individuals survive in the wild in the location where introduced, reproduction occurring but population not self-sustaining) using the Unified Framework for Biological Invasions (Blackburn et al. 2014). *Acacia floribunda* individuals of this species had galls on them (formed by Trichilogaster acaciaelongifoliae, see McGeoch & Wossler 2000) and no seeds were observed, therefore it was classed as C1 (individuals surviving in the wild in location where introduced, no reproduction). No plants of *Acacia retinodes* were found, although the species is known to occur in Tokai, in the Western Cape. It was clear from this study that more work was needed.

My study comprises a systematic and detailed approach to assess the invasiveness and the potential for eradication of species with very limited distribution in South Africa that have not as yet been studied in detail (*Acacia adunca*, *A. cultriformis, A. fimbriata*, *A. piligera, A. retinodes*, and *A. viscidula*) as well as other *Acacia* species that are found to be naturalised in Chapter 2. Several studies have shown that reproductive traits have been associated with the success of invasion (Richardson & Kluge, 2008; Correia 2014; Gibson et al. 2011). Thus, understanding the seed ecology of Australian acacias can provide good insights into their invasive potential and contribute to better management strategies. The reproductive ecology of many invasive Australian acacias have been well studied and documented (Richardson and Kluge 2008; 2010; Gibson et al. 2011; Strydom et al. 2011;2017 and this has helped in the progress made in managing invasive species in South Africa. Understanding the seed bank ecology of Australian *Acacia* species is very important before attempting eradication; Correia (2014) indicated that large amounts of long-lived and highly viable seeds may make it impossible to achieve eradication. Thus, it is important to determine seed viability and seed bank size.

The aims of the thesis was to determine:

- how many Australian *Acacia* species have been introduced to South Africa (Chapter 2);
- which species are still present and what is their status (Chapter 2);
- the potential to eradicate naturalised wattles from South Africa (Chapter 3); and
- provide recommendations for listing of and management strategies for wattles (Chapters 2,3 & 4).

Chapter 2: Even well studied groups of alien species are poorly inventoried: Australian *Acacia* species in South Africa as a case study

Submitted to the journal *Biological Invasions*

Author contributions:

Nkoliso Magona, David M Richardson & John R Wilson: Planned the study

Nkoliso Magona: Collected data, did all statistical analyses and wrote the first draft

David M Richardson & John R Wilson: Edited the manuscript

Suzaan Kritzinger-Klopper: assisted with field work

John R Wilson: Provided guidance

Abstract

Understanding the status and extent of alien plants is crucial for effective management. I explore this issue using Australian *Acacia* species (wattles) in South Africa (a global hotspot for wattle introductions and tree invasions). The last detailed inventory of wattles in South Africa was based on data collated forty years ago. This paper aims to determine: 1) how many Australian *Acacia* species have been introduced to South Africa; 2) which species are still present; and 3) the status of naturalised taxa that might be viable eradication targets. All herbaria in South Africa with specimens of introduced Australian *Acacia* species were visited and locality records were compared with records from the literature, various databases, and expert knowledge. For taxa not already known to be widespread invaders, field surveys were conducted to determine whether plants are still present, and detailed surveys were undertaken of all naturalised populations. For all naturalised taxa I also sequenced one nuclear and one chloroplast gene to confirm their putative identities. I found evidence that 114 Australian *Acacia* species are reported to have been introduced to South Africa (an increase of 60% from previous work), but I could confirm the presence of only 50 species. Seventeen wattle species are invasive (16 are in category E and one in category D2 in the unified framework for biological invasions); eight have naturalised (C3); and 25 are present but were not found to be producing viable seed (C1). DNA barcoding did not provide conclusive identifications for all taxa assessed, but helped to identify four species not previously recorded in South Africa. Given the omissions and errors found during this systematic re-evaluation of historical records; it is clear that analyses of the type conducted here are crucial if the status of even well studied groups of alien taxa is to be accurately determined.

Keywords: Biological invasions, herbaria, inventory, invasive species, management plan, tree invasions

2.1 Introduction

Every country needs up-to-date lists of introduced species to ensure that management actions are directed appropriately to deal with taxa at all stages of the introduction-naturalization-invasion continuum (Latombe et al. 2017; McGeoch et al. 2012; Regan et al. 2002). Several types of errors and biases typically exist in such species lists. These include: insufficient survey information, inappropriate data resolution, undocumented data, inaccessible data, lack of sufficient information on native range distribution, incomplete information, misidentifications, synonyms, and un-described species (Regan et al. 2002; McGeoch et al. 2012; Jacobs et al. 2017). For plants, sources of these errors and biases in the published literature, in museums, and in herbaria needs to be assessed to create more comprehensive, accurate and reliable databases to inform management.

Australian *Acacia* species (wattles) are a good group to address the dimensions of these problems because: 1) introductions and plantings of species in this group have been fairly well documented; 2) wattles are among the most widely transferred tree species and well-studied invasive plant species in the world; and 3) wattles are often a priority for management (Marais et al. 2004), given the substantial negative impacts they can cause and the difficulties of controlling established invasions (Wilson et al. 2011).

Wattles have been introduced to many parts of the world for many purposes (Le Maître et al. 2002; Kull and Tassin 2012), and they have played a major role in improving the livelihoods of communities (Kull et al. 2011; van Wilgen et al. 2011) and in economic growth (Griffin et al., 2011; Richardson et al. 2011). Despite these benefits, some wattle species have also become widespread invaders, threatening biodiversity by transforming ecosystems (Le Maître et al. 2000, 2011; Richardson and Van Wilgen 2011).

There are approximately 1022 Australian *Acacia* species (formerly grouped in *Acacia* subgenus Phyllodineae), of which at least 38% are known to have been moved by humans to areas outside their native ranges, at least 71 have become naturalized, and at least 23 have become invasive (i.e. have spread over substantial distances from planting sites) (Richardson et al. 2011; Rejmánek and Richardson 2013).

Knowledge of the introduction history of these species is crucial for understanding and predicting their performance (Wilson et al., 2011), and to guide management strategies (van Wilgen et al. 2011). The long history of introductions and widespread dissemination of Australian *Acacia* species around the world has created opportunities to investigate factors that drive the success and failure of introductions, and to determine how native species respond to such events (Castro-Díez et al. 2011; Richardson et al. 2011).

South Africa has a long history of wattle introductions. Several species (notably *A. cyclops*, *A. longifolia* and *A. saligna*) were introduced in the early 18th century by the Cape Colonial Secretary to stabilise dunes near Cape Town (Ross 1975; Poynton 2009); and a few decades later several species, e.g. *A. decurrens, A. mearnsii*, and *A. melanoxylon*, were introduced for timber production (Poynton, 2009). Where these species were planted for forestry, native vegetation was removed to allow the acacias to establish without competition (Richardson and Rejmánek 2011). In the early 19th century, several other species were introduced for ornamental purposes, e.g. *A. baileyana*, *A. elata*, and *A. podalyriifolia* (Donaldson et al. 2014a, b). As a result of this long and varied history, South Africa possibly has the greatest diversity of Australian *Acacia* species introductions and the most widespread wattle invasions of anywhere in the world (Richardson et al. 2011; Richardson and Rejmánek 2011; Rejmánek and Richardson 2013).

The history of wattle species introduced and planted for forestry purposes in South Africa was reviewed by R.J. Poynton (2009). However, the information on which this assessment was based was collated in the 1970s and now needs updating. For example, recent surveys have shown that some species are much more abundant and widespread than previously thought (e.g. *A. paradoxa*; Zenni et al. 2009), and several species that were not listed by Poynton (2009) are now invasive (e.g. *A. stricta*; Kaplan et al. 2014).

Despite several decades of intensive management of invasive wattles in South Africa (van Wilgen et al. 2011), we know little about species other than those with substantial commercial value and those that are well-established invaders. What is known, however, is that invasions of Australian *Acacia* species are still increasing in geographical extent, abundance, and magnitude of impact (Henderson and Wilson 2017). Even the most widespread invasive species have not reached all potentially invasible sites (Rouget et al. 2004), and many naturalised species began spreading recently (e.g. Zenni et al. 2009; Kaplan et al. 2012, 2014). Rouget et al. (2016) quantified different aspects of this “invasion debt” for wattles, and found that southern Africa has a large invasion debt. Invasion debt is the time delayed invasion of species introduced (Rouget et al. 2016) If the invasion debt were realised, there will be a substantial escalation in the overall ecological and economic impacts of wattles (Richardson et al. 2015). This means that there is a need to act before these species start to spread to other places. If the widespread and invasive species have not reach their full invasiveness yet, this means that species with limited distribution are yet to become widespread.

Richardson et al. (2011) reported that about 70 species of Australian *Acacia* species are known to have been introduced to South Africa, some as early as the 1830s (Adamson, 1938; Poynton, 2009). Sixteen species are currently considered invasive in the country (Rejmánek and Richardson 2013). There are also records of naturalized populations of *A. adunca, A. cultriformis, A. fimbriata, A. pendula, A. viscidula,* (Wilson et al. 2011; van Wilgen et al. 2011) and there are localized populations of *A. retinodes* and *A. ulicifolia* (Wilson et al. 2011; van Wilgen et al. 2011). However, the identification of these species remains to be verified, and the status of other species reported in the country is unknown. In this context, this study set out to determine: 1) how many Australian *Acacia* species have been introduced to South Africa; 2) which species are still present and their status?; and 3) what is the extent of naturalised populations.

2.2. Methods

2.2.1. Creating a list of species that have been introduced into South Africa

I reviewed formal literature sources, student theses, and unpublished records for records of Australian acacias. All relevant herbaria, museums, and botanical gardens in South Africa with specimens or collections of Australian *Acacia* species were also visited or consulted. Literature and online data bases were searched using the genus and species name as search terms to collate information on specimens from other herbaria around the world that were previously recorded in South Africa. The dataset was expanded with data from other sources that list introduced species distributions in southern Africa, including: 1) the Southern African Plant Invaders Atlas (SAPIA, Henderson and Wilson 2017); 2) I-Spot (http://www.ispot.org.za/); and 3) the National Herbarium Computerized Information System (PRECIS online database http://posa.sanbi.org/intro_precis.php; Morris and Glen, 1978). Locality records from herbaria data were compared with records in the literature, databases and experts to obtain updated locality records. Data collected from different sources were filtered and duplicates were removed.

During herbaria visits I followed a standard protocol for dealing with records of Australian acacias (Fig. 2.1). Records with precise coordinates were noted and added to the locality list. Google Earth was used to find the likely locality of the *Acacia* plants. Landowners and managers were contacted, and field surveys were conducted to search for plants. For records with imprecise locality description and no coordinates, the source of the record was consulted.

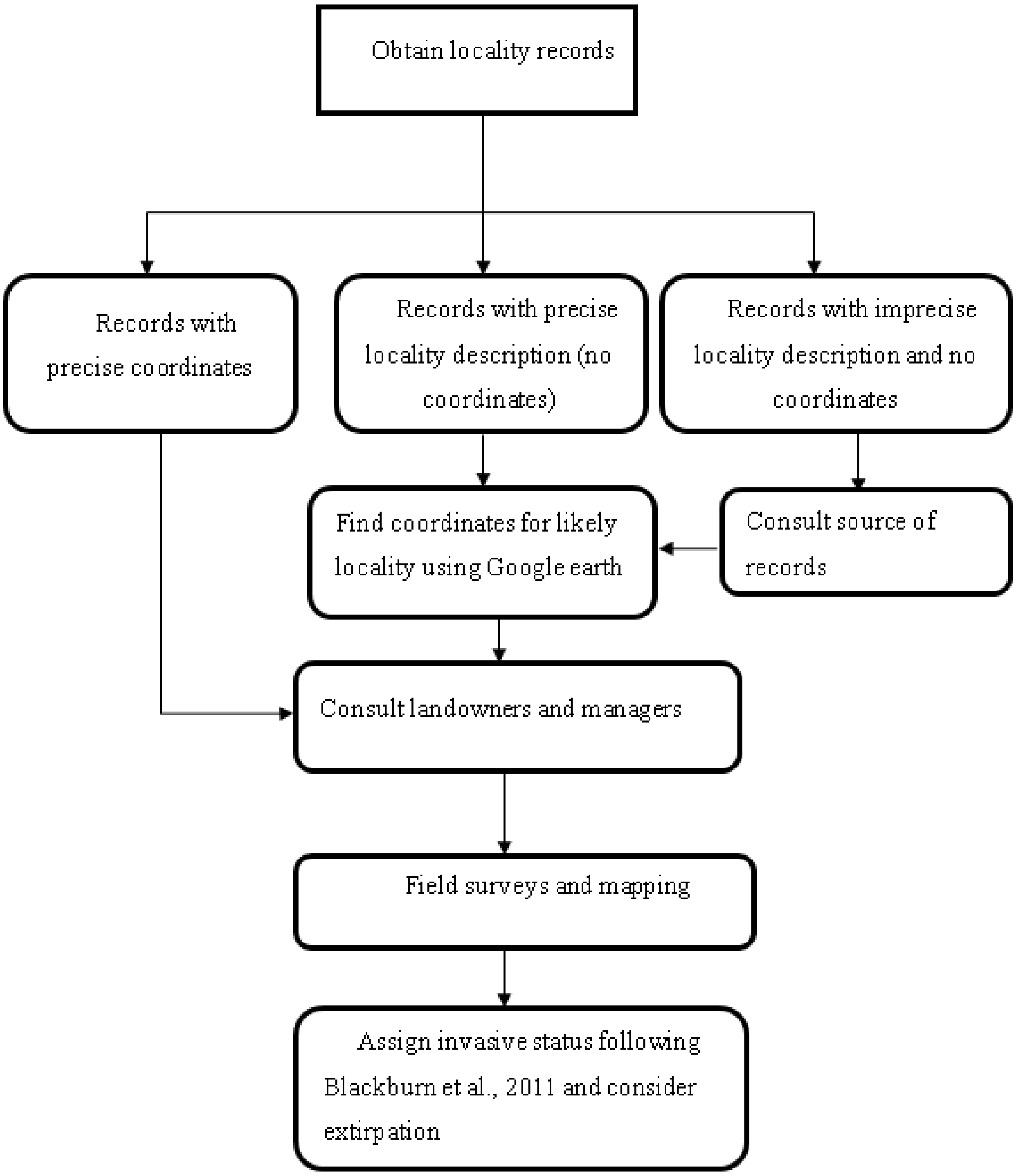


Figure: 2.1. The protocol used in this paper for dealing with records of Australian *Acacia* species in South Africa. The protocol resulted both in an inventory of species in South Africa, and recommendations for incursion response.

2.2.2. Determining which species are still present

After compiling the list of introduction sites of wattles in South Africa, I conducted field surveys to confirm whether species were still present. I also specifically looked for locations where many species had been cultivated (e.g. arboreta and experimental plantings) to determine whether other taxa that have not been formally recorded were present. In cases where a location was provided but precise co-ordinates were not given, I consulted relevant officials (e.g. local conservation officers).

When comparing different lists it was also possible to determine the types of errors (e.g. human error and species identification) in the lists (e.g. Jacobs et al. 2017). To this end, I checked the identities of 59 herbarium records. Many *Acacia* species are morphologically very similar which makes it difficult to identify some taxa based on morphology alone. If the identity of a taxon collected in the field was not known, or if the identity of a taxon had not previously been confirmed using a molecular approach, I used a DNA sequencing approach to verify identities. I sequenced two gene regions, the plastid psbA-trnH intergenic spacer and the nuclear external transcribed spacer region (ETS), for comparison against existing molecular data (Miller et al. 2016). DNA were extracted from silica-dried leaf material from selected taxa (Supplementary Table 2.1) using the cetyltrimethylammonium bromide (CTAB) method as described by Doyle and Doyle (1990). psbA-trnH was amplified using the primers psbA (5'-GTT ATG CAT GAA CGT AAT GCT C-3') and trnH(GUG) (5'-CGC GCA TGG ATT CAC AAT CC-3') and the following polymerase chain reaction (PCR) conditions: Initial denaturation at 80 °C for 5 min; followed by 35 cycles of denaturation at 94 °C for 30 sec, annealing at 60 °C for 30 sec, and extension at 72 °C for 1 min. A final elongation step was done at 72 °C for 10 min. Each 30 µl reaction contained ca. 300 ng of genomic DNA, 200 µM of each dNTP (Thermo Scientific, supplied by Inqaba Biotec, Pretoria, South Africa), 10 pmoles of each primer, 0.3 U Taq DNA polymerase (Kapa Biosystems, supplied by Lasec, Cape Town, South Africa), PCR reaction buffer and 2 mM $MgCl_2$.ETS genes were amplified using the primers ATS-AcR2 (5'-GGG CGT GTG AGT GGT GTT TGG-3') and ETS-18S-IGS (5'-CAC ATG CAT GGC TTA ATC TTT G-3') and the following PCR conditions: Initial denaturation at 94°C for 3 min; followed by 30 cycles of denaturation at 94 °C for 60 sec, annealing at 60 °C for 60 sec, and extension at 72 °C for 2 min. A final elongation step was done at 72 °C for 10 min. Each 30 µl reaction contained ca. 300 ng of genomic DNA, 200 µM of each dNTP (Thermo Scientific, supplied by Inqaba Biotec, Pretoria, South Africa), 10 pmoles of each primer, 0.3 U Taq DNA polymerase (Kapa Biosystems, supplied by Lasec, Cape Town, South Africa), PCR reaction buffer and 1.25 mM MgCl2.PCR products for both gene regions were purified using the QIAquick® PCR Purification Kit (Qiagen, supplied by White Head Scientific, Cape Town, South Africa) and sequenced using the ABIPRISM BigDye Terminator Cycle Sequencing Ready Reaction kit and an automated ABI PRISM 377XL DNA sequencer(PE Applied Biosystems, Foster City, CA, USA). DNA sequence data were aligned and edited using bio edit version 7.0.5.3 (Hall, 1999) followed by manual editing. Individual gene sequences were blasted against the NCBI's GenBank database (http://blast.ncbi.nlm.nih.gov/Blast).^^^

2.2.3. The introduction status of *Acacia* species present in South Africa

The observed populations of *Acacia* species were assigned an introduction status following the unified framework for biological invasions (Appendix A; Blackburn et al. 2011), as interpreted and elucidated for trees by Wilson et al. (2014). I conducted field surveys to search for species at previously known or recorded sites obtained from herbarium records and the literature. Google Earth and Google Street View were used to initially search for trees using the geographic coordinates on herbarium records [see Visser et al. (2014) for discussion on the use of Google Earth in the study of tree invasions]. This was useful for preparing for surveys and for initial work. For all plants found during field surveys, I measured: plant canopy dimensions, height, stem, basal diameter, presence/absence of reproductive structures. I asked informed members of the community where the plants were found and whether they had seen seedlings under these trees. To investigate the presence of a soil seed-bank, several soil cores were taken at each site (N. Magona, unpubl. data). To estimate the total seed population, a square grid (25m x 25m) covering the densest part of the population was set up for A. adunca, A. fimbriata, A. piligera and A. viscidula. The grid was split into 5 x 5 m cells, and a soil sample was collected using a cylindrical soil corer (15cm deep and 7 cm in diameter) in each cell (giving 25 samples per grid). My sampling method was similar to grid method that Strydom et al. (2011) used. However, Strydom et al. (2011) indicated that a grid method is not suitable for a large population or area as it might miss spatial variation in the seedbank but, all the species I was working with had relatively small area hence, I used this method and I did found high number of seedbank for all the species. A summary of the status of each naturalised populations was prepared following the recommendations of Wilson et al. (2014).

2.3. Results

I found evidence that 114 Australian *Acacia* species have been introduced to South Africa (Table 2.1). Of these, I could confirm the presence of only 50 species (Fig. 2.2). In terms of Blackburn et al.'s (2011) Unified Framework for Biological Invasions (see Appendix 2A for a full description of the categories), 16 of these species are in category E and one *(A. fimbriata*) is in category D3 (i.e. there are 17 invasive species). Eight species are naturalized but not yet invasive (category C3). I found no evidence that the remaining 25 species have produced viable seed in South Africa; these taxa thus fall in category C1. Status reports on the six naturalised species are presented in Appendices 2.2–2.7.

Table 2.1: The status of Australian *Acacia* species in South Africa based on historical records, field sampling, and DNA barcoding.

Acacia species [authorities given from original source]	Number of herbarium records	Population size	Location	Status[1]	Number of records in SAPIA[2]	QDGCs occupied in SA[3]	GenBank accession numbers for ETS and *psb*A-*trn*H
A. acinacea Lindl.	2	NA	Cape Peninsula	Not re-found	not listed	NA	
A. acuaria W. Fitzg	1	NA	University of Pretoria	Not re-found	not listed	NA	
A. acuminata Benth.	3	NA	Paarl div, Uitenhage div, Knysna, Stutterheim div, Robertson, Lichtenburg	Not re-found	not listed	NA	
A. adunca A. Cunn. ex G. Don	2	>100 plants	Paarl	C3	1	1	pending
A. alata R. Br.		NA	Johannesburg	Not re-found	not listed	NA	
A. ampliceps Maslin	0	~25 plants	Malmesbury	B2	not listed	NA	pending
A. ancistrocarpa x *arida*	0	~25 plants	Malmesbury	C3	not listed	NA	pending
A. aneura F. v. Muell.	1	~25 Plants	Zoutpansberg, Lichtenburg, Zoutpansberg	Not re-found	not listed	NA	pending
A. arenaria Schinz	1	NA	Pretoria	Not re-found	not listed	NA	
A. argyrophylla Hook.	1	NA	Johannesburg	Not re-found	not listed	NA	
A. aspera Lindl.	1	NA	Pretoria	Not re-found	not listed	NA	
A. aulacocarpa A.Cunn. ex Benth.	0	NA		Not re-found	not listed	NA	
A. auriculiformis A.Cunn. ex Benth	0	~25 Plants	Malmesbury	Not re-found	not listed	NA	pending
A. baileyana F. v. Muell.	Many	Many	multiple	Not re-found	184	101	JX572184.1
A. bidwillii Benth	0	~25 plants	Malmesbury	Not re-found	not listed	NA	pending
A. birnevata DC.	1	NA	Cape Peninsula, Pretoria, Johannesburg	Not re-found	not listed	NA	
A. bivenosa DC.		~25 Plants	Malmesbury	Not re-found	not listed	NA	pending
A. brachyobotrya Benth.	2	NA		Not re-found	not listed	NA	
A. brachystachya Benth.	2	~25 Plants	Pretoria	B2	not listed	NA	pending
A. burrowii Maiden	0	~25 Plants	Malmesbury	B2	not listed	NA	pending
A. calamifolia sweet ex Lindt	2	NA	Pretoria	Not re-found	not listed	NA	

Acacia species [authorities given from original source]	Number of herbarium records	Population size	Location	Status[1]	Number of records in SAPIA[2]	QDGCs occupied in SA[3]	GenBank accession numbers for ETS and *psb*A-*trn*H
A. calcicola Forde & Ising	0	~25 Plants	Malmesbury	B2	not listed	NA	pending
A. cambagei R.T.Baker	0	~25 Plants	Malmesbury	B2	not listed	NA	pending
A. cardiophylla A. Cunn. ex Benth.	4	NA	Johannesburg, Pretoria	Not re-found	not listed	NA	
A. celastrifolia Benth.	0	NA	University of Pretoria	Not re-found	not listed	NA	
A. cognata Domin	1	NA	Pretoria	Not re-found	not listed	NA	
A. colei Maslin & L. A. J. Thomson	0	~25 Plants	Malmesbury	C3	not listed	NA	pending
A. cowleana Tate.	0	NA		Not re-found	not listed	NA	
A. crassicarpa A. Cunn. ex Benth.	0	NA		Not re-found	not listed	NA	
A. cultriformis A. Cunn. ex G.Don	10	~50 Plants	Pretoria, Johannesburg, Middelburg, Grahamstown	C3	1	1	pending
A. cyclops A. Cunn. ex G. Don	Many	Many	Multiple	E	1282	172	JF277064.1
A. dealbata Link	Many	Many	Multiple	E	1667	299	
A. deanei (R.T. Bak.) Welch, Coombs & McGlyn	3	NA	Pretoria	Not re-found	not listed	NA	
A. decora Reichb.	3	NA	Albany Div.	Not re-found	not listed	NA	
A. difficilis Maiden	0	~25 Plants		B2	not listed	NA	
A. decurrens Willd	Many	Many	Multiple	E	341	124	
A. dodonaeifolia (Pers.) Balb.	1	NA		Not re-found	not listed	NA	
A. doratoxylon A.Cunn.	2	NA	Cape Peninsula	Not re-found	not listed	NA	
A. drummondii Lindl.	1	NA	University of Pretoria	Not re-found	not listed	NA	
A. elechantha M. W. McDonald & Maslin	0	~25 Plants	Malmesbury	B2	not listed	NA	pending
A. elongata Sieber	1	NA	Pretoria	Not re-found	not listed	NA	
A. extensa Lindl.	2	NA	Johannesburg	Not re-found	not listed	NA	
A. falciformis DC.	0	NA		Not re-found	not listed	NA	

Acacia species [authorities given from original source]	Number of herbarium records	Population size	Location	Status[1]	Number of records in SAPIA[2]	QDGCs occupied in SA[3]	GenBank accession numbers for ETS and *psbA-trnH*
A. elata A.Cunn. ex Benth.	Many	Many	Multiple	E	99	48	JX572190.1
A. fimbriata A. Cunn. ex G. Don	4	>2000 Plants	Grahamstown	D2	1	1	Pending
A. flexifolia A. Cunn. ExBenth.	1	NA	Johannesburg	Not re-found	not listed	NA	
A. flocktoniae Maiden	1	NA	Pretoria, Johannesburg	Not re-found	not listed	NA	
A. floribunda (J.C. Wendl.) Willd.	3	>6 Plants	Johannesburg; Pretoria; Bloemfontein	C1	not listed	NA	
A. glaucescens Willd.	1	NA	Pretoria	Not re-found	not listed	NA	
A. harpophylla F.Muell. ex Benth.	0	~25 Plants	Malmesbury	B2	not listed	NA	Pending
A. hemsleyii Maiden	0	~25 Plants	Malmesbury	B2	not listed	NA	Pending
A. holosericea A.Cunn. ex G.Don	0	~25 Plants	Malmesbury	B2	not listed	NA	Pending
A. howittii F.Muell.	1	NA	Albany Div.	Not re-found	not listed	NA	
A. implexa Benth	11	Many	Stellenbosch, Tokai, Wolseley	E	3	3	
A. iteaphylla F.J. Muell.	2	NA	Pretoria	Not re-found	not listed	NA	
A. ixiophylla Benth.	2	NA	Johannesburg	Not re-found	not listed	NA	
A. jonesi F. v. Muell. & Maides	1	NA	Pretoria	Not re-found	not listed	NA	
A. julifera Benth	0	~25 Plants	Malmesbury	B2	not listed	NA	Pending
A. kempeana F.Muel.	1	NA		Not re-found	not listed	NA	
A. lanigera A. Cunn.	1	NA	Lydenburg dist.	Not re-found	not listed	NA	
A. latipes Benth	1	NA	Addo Elephant National Park	Not re-found	not listed	NA	
A. leptocarpa A. Cunn. ex Benth.	0	~25 Plants	Malmesbury	B2	not listed	NA	Pending
A. leptoneura Benth.	2	NA	Pretoria	Not re-found	not listed	NA	
A. leptospermoides Benth.	1	NA	Pretoria	Not re-found	not listed	NA	
A. ligulata A.Cunn. ex Benth.	1	NA	Malmesbury	B2	not listed	NA	Pending
A. linearis (H. Wendl.) Macbr.	1	NA	Pretoria	Not re-found	not listed	NA	

Acacia species [authorities given from original source]	Number of herbarium records	Population size	Location	Status[1]	Number of records in SAPIA[2]	QDGCs occupied in SA[3]	GenBank accession numbers for ETS and *psb*A-*trn*H
A. lineolate Benth		NA		Not re-found	not listed	NA	
A. longifolia (Andr.) Willd.	Many	Many	multiple	E	446	97	
A. maconochieana Pedley	0	NA	Malmesbury	C3	not listed	NA	Pending
A. macradenia Benth.	3	NA	Cape Peninsula	Not re-found	not listed	NA	
A. mangium Willd.	0	1 tree	Malmesbury	B2	not listed	NA	Pending
*A. mearnsii*de Willd.	Many	Many	multiple	E	4313	462	JX572209.1
A. melanoxylon R. Br.	Many	Many	multiple	E	678	167	KJ782179.1
A. monticola J. M. Black	0	~25 Plants	Malmesbury	B2	not listed	NA	Pending
A. murrayana F. Muell. ex Benth.	0	~25 Plants	Malmesbury	B2	not listed	NA	Pending
A. myrtifolia (Sm.) Willd.	3	NA	Johannesburg, Pretoria	Not re-found	not listed	NA	
A. neriifolia Cunn.	3	NA	Pretoria, Germiston	Not re-found	not listed	NA	
A. oxycedrus Sieber ex. DC	1	NA	Pretoria	Not re-found	not listed	NA	
A. paradoxa DC	1	C3	Devils Peak, Table Mountain, Cape Town	D2	4	2	
A. pendula A. Cunn.	4	C1	Middelburg, Excelsior dist. Delareyville, Lichtenburg, Bloemhof, Kroonstad dist.,Beaufort West	C1	not listed	NA	
A. pernninervis Sieb.	3	NA	Cape Peninsula	Not re-found	not listed	NA	
A. piligera A. Cunn.	0	>100	Tokai	C3	not listed	NA	Pending
A. podalyriifolia A. Cunn. ex G. Don	Many	Many	multiple	E	159	78	JX970902.1
A. pravissima F. v. Muell.	1	NA	Pretoria	Not re-found	not listed	NA	
A. prominens A. Cunn. ex G. Don	1	NA	Pietermaritzburg, Zoutpansberg, Centurion	Not re-found	not listed	NA	
A. pruinocarpa Tindale	0	~25 Plants	Malmesbury	B2	not listed	NA	Pending
A. pruinosa A. Cunn. ExBenth.	4	NA	Cape Peninsula	Not re-found	not listed	NA	
A. pycnantha Benth.	Many	Many	multiple	E	182	38	KC261818.1
A. quornensis Black	2	NA	Johannesburg	Not re-found	not listed	NA	

Acacia species [authorities given from original source]	Number of herbarium records	Population size	Location	Status[1]	Number of records in SAPIA[2]	QDGCs occupied in SA[3]	GenBank accession numbers for ETS and *psb*A-*trn*H
A. retinodes Schlechtd.	4	>100 Plants	Pretoria dist., Stellenbosch, Johannesburg, Tokai	C3	not listed	NA	
A. richii A.Gray	1	NA	Pretoria	Not re-found	not listed	NA	
A. rubida A. Cunn.	1	NA	Middelburg dist.	Not re-found	not listed	NA	
A. saliciformis Tindale	1	NA	Pretoria	Not re-found	not listed	NA	
A. salicina Lindl.	0	~35 Plants	Lüderitz south, Johannesburg, Gwelo	B2	not listed	NA	
A. saligna (Labill.) H.L. Wendl.	Many	Many	Multiple	E	1302	164	KM095754.1
A. schinoides Benth	1	NA	Stellenbosch	Not re-found	not listed	NA	
A. scirpifolia Meisn.	2	NA	Paarl div.	Not re-found	not listed	NA	
A. sclerosperma F.Muel.	0	~25 Plants	Malmesbury	B2	not listed	NA	Pending
A. spectabilis A. Cunn.	0	NA	Johannesburg	Not re-found	not listed	NA	
A. stricta (Andrews) Willd.	1	Many	Knysna	E	6	6	
A. squamata Lindl.	1	NA	Suurberg Nature Reserve	Not re-found	not listed	NA	
A. stenophylla Malme	0	>25 Plants	Malmesbury	B2	not listed	NA	Pending
A. subporosa F.Muell.	1	NA	Cape Peninsula	Not re-found	not listed	NA	
A. tumida F. Muell. ex Benth.	0	NA	Malmesbury	B2	not listed	NA	Pending
A. ulicifolia (Salisb.) Court var. *brownei* (Poir.) Pedlez	1	Very scarce	Pretoria Cape Peninsula, Transkei -	C1	not listed	NA	
A. ulicina Meisn.	1	NA	Pretoria	Not re-found	not listed	NA	
A. uncifera Benth.	1	NA	Pretoria	Not re-found	not listed	NA	
A. undulifolia A.*Cunn.*	1	>100 Plants	Cape Peninsula	Not re-found	not listed	NA	
A. victoriae Benth.	0	NA	Malmesbury	Not re-found	not listed	NA	
A.viscidula A. Cunn. ExBenth.	2	>100 Plants	Pretoria, Grahamstown	C3	1	1	
A. verniciflua A. Cunn	1	NA	Pretoria	Not re-found	not listed	NA	
A. verticillata (L'Her.) Willd.	0	NA	Pretoria	Not re-found	not listed	NA	

Acacia species [authorities given from original source]	Number of herbarium records	Population size	Location	Status[1]	Number of records in SAPIA[2]	QDGCs occupied in SA[3]	GenBank accession numbers for ETS and *psb*A-*trn*H
A. visite Ker-Gawler	0	3 Plants	University of Free State	C1	not listed	NA	
A. wildenowiana H.L.Wendl.	1	NA	Addo Elephant National Park	Not re-found	not listed	NA	
A. xiphophylla E.Pritz.	0	~25 Plants	Malmesbury	B2	not listed	NA	Pending

[1]Status is as per the Unified Framework for Biological Invasions (Blackburn et al. 2011; See Appendix A for details), with “Not re-found” means that records exist from botanical gardens or experimental plantings but could not be found at recorded localities.
[2]Number of records of naturalised populations in the Southern African Plant Invader Atlas (SAPIA) as of January 2017.
[3]The number of quarter-degree grid cells occupied (QDGCs) in South Africa (from SAPIA). Each QDGC is 630–710 km^2 at the latitude of South Africa).

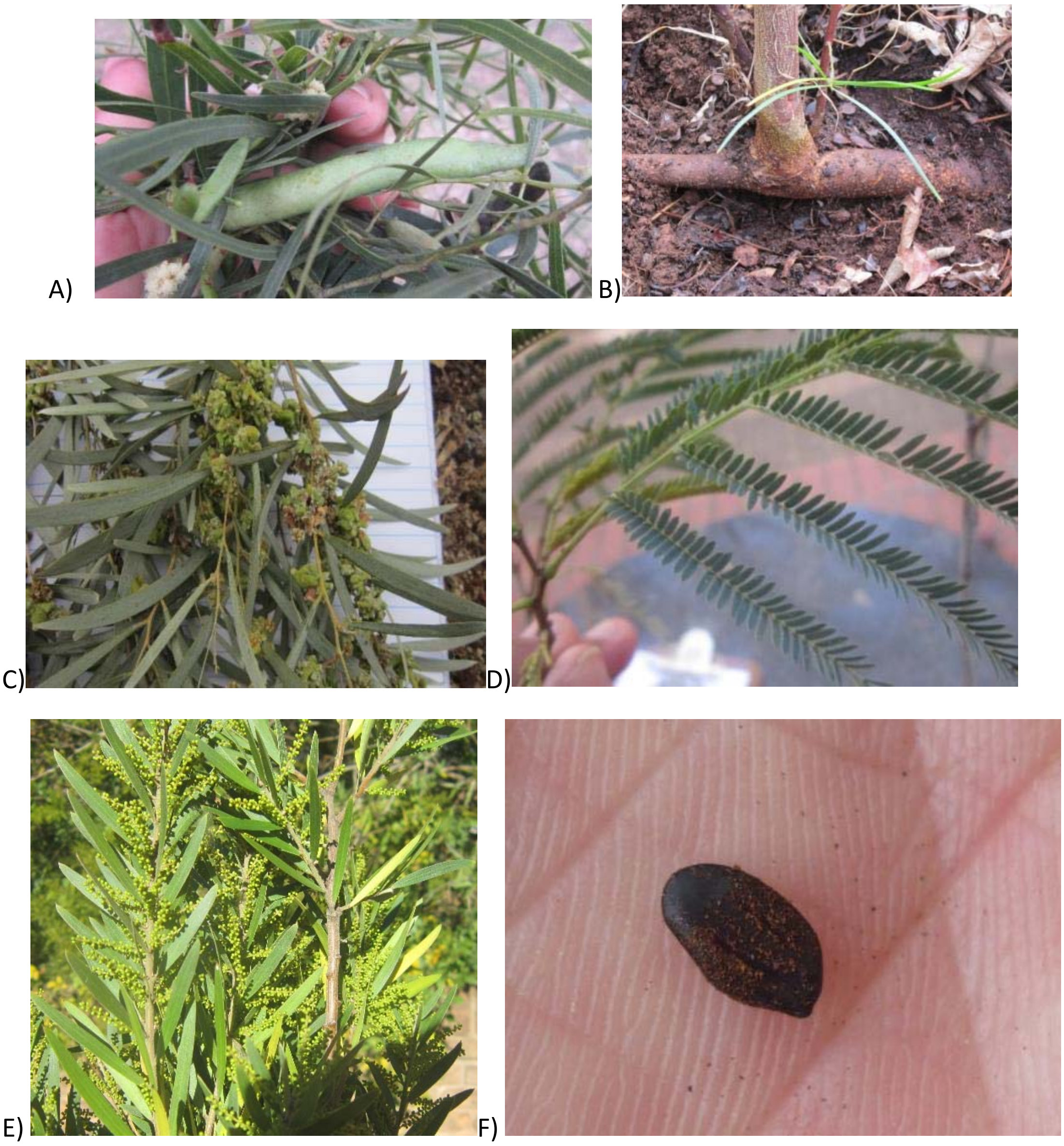


Figure 2.2: Examples of Australian *Acacia* species found in this study. A) *Acacia salicina* with green pods in the Johannesburg Botanical Gardens; B) *A. viscidula* root sucker in a naturalised population in Newlands, Cape Town; C) *A. pendula* in Bloemfontein showing galls formed by the biological agent *Dasineura dielsi* (which was released to control *A. cyclops*); D) *A. visite* with bi-pinnate phyllodes from the University of the Free State; E) A planted individual of *A. floribunda* showing phyllodes and flower-spikes in Johannesburg; F) A seed of *A. piligera* collected at Tokai, Cape Town. Photos: Nkoliso Magona.

The 114 species found in this study represent a ~60% increase on the previous estimate of 70 species (Richardson et al. 2011). These additional species include taxa not previously known from outside Australia (*A. aquaria, A. latipes, A. leptospermoides, A. saliciformis, A. ulicina*, and *A. uncifera*; Richardson et al. 2011).

I found a few errors on herbaria specimen labels: three instances of misspelled or incorrect species names (see Table 2.2). However, in old reports, publications, and species lists there were seven noticeable instances where species names were incorrectly assigned or were misspelt (*A. aculeatissima* instead of *A. ulicifolia*, *A. aulacorpa*, instead of *A. aulacocarpa, A. drummardii* instead of *A. drummondii*, *A. koa* instead of *A. floribunda, A. ulicifolium* instead of *A. ulicifolia A. iteaphylla* instead of *A. itheaphylla, A. verticillata* instead of *A. verticulata*).

Table: 2.2. Methodology followed in determining errors in lists of *Acacia* species in herbaria and in the literature.

Errors	Explanatory questions	Method	Results
Human error (species misidentification, synonyms)	How many species had been misidentified?	All herbarium specimens of *Acacia* species were examined for correct identification. If it was suspected that a specimen had been misidentified, the identification was verified using identification guides (e.g. online database, reference books), experts or molecular DNA barcoding if necessary. The total number of herbarium vouchers examined and misidentifications were counted. Furthermore, any known cases of species being misidentified in the literature was noted.	Only one species had been misidentified: *A. koa* as *A. floribunda*
	How many species had been incorrectly named (synonyms and incorrect spelling)?	A search was conducted of the literature and online databases to determine the total number of *Acacia* species which had their names changed. When examining herbarium specimens, the number of times the records had been renamed (i.e. old names crossed out and new names recorded) was counted. To determine the number of times *Acacia* species have had their names changed, the literature and databases (www.theplantlist.org) was used. The Plant List (www.theplantlist.org) was used as the source of recognized names. The number of records using old names (not the currently accepted name) were counted.	Five species names were misspelled: *A. aulacocarpa as A. aulocarpa*; *A. drummondii* as *A. drummardii*; *A. ulicifolia as A. ulicifolium*; *A. iteaphylla as A. itheaphylla;* *A. verticillata as A. verticulata.*
Which errors have been perpetuated?		The identified errors were assessed for presence in multiple data sources to determine whether an error has been repeated. The primary source of the identified errors was also assessed by conducting literature search using the specific error as search term.	No errors found in any database

Errors	Explanatory questions	Method	Results
Resolution of data and scaling of “alien range”	For how many records was the resolution of data too coarse to be useful?	Field surveys were conducted on reported population localities from SAPIA, herbaria and literature. The number of records for which the resolution of data (e.g. quarter-degree grid cell, town or region) was too course to allow individuals to be located was recorded. The data from SAPIA, herbaria and literature was compared with the survey results to provide a fine resolution locality	Using historical data was not accurate as the resolution was too coarse (recorded at the scale of quarter-degree cells). Using such data was unreliable for locating and assessing the extent of species spread. I mapped the species at finer scales to avoid such issues.
Data and knowledge not documented	How many records not documented?	New locality records were followed up in field surveys to establish the current status of species localities. The number of records that are only the result of undocumented expert knowledge and surveys were counted. Furthermore, some species identification flyers were distributed in surveyed areas to solicit new species sightings. Any new sightings resulting from the public sighting were counted.	Two localities found.26 *Acacia* species were recorded at Damara farm and one species at the University of the Free State.

Reference data for one or both genes for voucher specimens of acacias that matched our putative species identifications were available for only 19 taxa out of the 54 for which I generated DNA sequencing data (Supplementary Table 2.1). For these DNA sequencing data and putative field identifications were in agreement for 11 accessions. Where DNA sequencing data were only available for one gene region for voucher specimens (Supplementary Table 2.1). I could reliably assign taxonomic affinities if there were high DNA sequence similarity (99-100%) with high statistical support for that gene regions and agreement with putative field identifications (e.g. *A. cultriformis*). Blast results with high DNA sequence similarity (99-100%) and statistical support also led to the discovery of *Acacia* species not previously recorded from South Africa. For many species I could not assign putative field identities based on morphological data. For these, DNA sequencing data for both gene regions identified, with high certainty, two taxa (*A. neriifolia* and *A. hakeoides*).

2.4. Discussion

Before this study, 70 Australian *Acacia* species were known to have been introduced to South Africa (Richardson et al. 2011). I found evidence that another 44 species had been introduced to the country. Of the revised list of 114 species for which records exist of introduction to, or presence in, South Africa (Table 2.1), I could confirm that at least 50 species are still present in the country. Thirty of these specimens were from experimental farms or botanical gardens and only seven of these could be traced to existing plantings. There were four major reasons for the discrepancy between the list of species recorded as introduced to South Africa and the list of species confirmed to be still present in the country. First, during the survey I came across an old experimental forestry trial set up to identify species suitable for dry-land agroforestry (Damara Farm in the Western Cape; see Supplementary Material 2.2). Twenty-nine Australian *Acacia* species were recorded on that farm, of which I could find 26. None of these taxa have naturalised.

Second, specimens of several species are present in the National Herbarium in Pretoria but had not been included in previous lists because the herbarium records had not yet been digitised. Additionally, a few listed species were initially misidentified (e.g. *A. floribunda* misidentified as *A. fimbriata*).

Third, species might no longer be present at a site. Many of the records (particular the undigitised herbarium records) were from historical forestry plantings. When I followed up, I found that many of these planting were no longer present—they had been transformed for infrastructure development, agriculture, or other forms of land use. Most cases where listed species are no longer present were within the municipal areas of the cities of Johannesburg and Pretoria that have been converted to stock farms. For example, all available records of *A. cultriformis* that were assessed in Gauteng province are now under various forms of agriculture, while several records of other species in Poynton (2009) referred to arboreta that no longer exist. Alternatively, species may not have survived at sites of initial introduction due to unfavourable climatic conditions or biotic pressures; Poynton (2009) noted that most introduced *Acacia* species were grown in trial plantations, many of which did not survive. Whatever the cause, I had to assume that such species are no longer present in South Africa (see supplementary Table 2.2).

Finally, it is possible that, despite our best efforts, our searches were inadequate to (re)locate some species. I suspect this is unlikely to be a major cause, as Australian *Acacia* species have been extensively studied and managed in South Africa, and the taxa are often quite distinctive from the native flora. Some "missing" species might feasibly be surviving in soil-stored seed banks (seeds of many wattle species can retain viability in the soil for several decades; Richardson & Kluge 2008). However, there may be other localities like Damara Farm where multiple species have been cultivated and potentially still exist. Poynton (2009) noted that many old trial plantations were left unmanaged due to the closure of forest stations, and records of these sites might not be reflected in the information sources that I consulted. Given that 73 herbaria specimens and many literature reports lacked detailed locality data (longitude and latitude coordinates), it is possible that I simply was not looking in the right place.

Whatever the reasons for discrepancies in past estimates of wattle invasions in South Africa, it is clear that there is a high invasion debt for Australian *Acacia* species in the country (Rouget et al. 2016). There is no quantified evidence that these species will become invasive but, the fact that there are species that are not documented and no status about their current extent raises concerns as Rouget et al. (2016) found that species introduced long time ago are only starting to become invasive. It is possible that these species were introduced into climatically unsuitable site and the fear now is what if these species escape to suitable sites. If this debt were paid, it would lead to a substantial escalation in the extent of invasions and overall ecological and economic impacts of the group (Richardson et al. 2015). There appear to be no clear set of life-history features, or syndromes of traits, that separate invasive from non-invasive *Acacia* species (Gibson et al. 2011), nor is there a clear phylogenetic signal of invasiveness in the genus (Miller et al. 2017). This suggests that factors associated with propagule pressure and residence time have been the dominant drivers of invasiveness in this genus in South Africa. This highlights the importance of dealing with nascent invaders before population sizes and spatial extent are sufficiently large to drive self-sustaining invasions.

One way of reducing this invasion debt is through proactive control, e.g. the detection, identification, assessment, and control of naturalised populations before they are widespread invaders. Some of the naturalised populations of Australian acacias in South Africa occur only at a few sites and so eradication is possible, but for some species, *A. cultriformis* specifically, it is likely that they are present at other locations that were not detected in this study. During the field visits in the cities of Bloemfontein and Johannesburg, people that had *A. cultriformis* in their gardens reported that this species was present in many gardens in neighbouring areas. As this species has been widely planted, it is likely that the seed bank and high climatic suitability (Motloung et al. 2014) could make it a high invasion risk (Wilson et al. 2011). Of the naturalised species that were detected in this study, *A. cultriformis* is the only one for which nation-wide eradication is likely to be infeasible (given the problems locating all horticultural plantings).

Some of the taxa might also have been prevent from spreading due to the impact of biological control agents released to target the widespread Australian *Acacia* species. In this study, the biological control agents *Dasineura dielsi* (target species: *A. cyclops*) and *Trichilogaster acaciaelongifoliae* (target species: *A. longifolia*) were observed on both A*. floribunda* and *A. pendula. Dasineura dielsi* has previously been recorded on *A. melanoxylon, A. longifolia, A. saligna,* and *A. implexa* (Impson et al. 2009; Kaplan et al. 2012). It is likely that the agents reduced seed production in these species, potentially reducing the rate of spread of populations, though I suspect it is unlikely that the agents resulted in the extirpation of any populations without any other management or land use change.

Unlike other taxonomic groups of alien plants, where there are many misidentified herbarium records (e.g. *Melaleuca spp*.; Jacobs et al. 2017), the majority of the wattle species encountered here were correctly identified (or at least there was congruency between the molecular and morphological identifications). However, our molecular approach could not resolve all taxonomic ambiguities, especially in cases where there was insufficient reference data for vouchers specimens (Parmentier et al. 2013) or short DNA sequence reads (Stoeckle et al. 2011). This makes differentiation between closely related species difficult. About 50% of putative species in our list remained unidentified as molecular and morphological data were insufficient. This could be because DNA sequencing data for the gene regions that I used are not available for many wattle species. One of the challenges I faced was to identify species based on barcoding alone, as many showed 100% DNA similarity to more than one taxon. I assumed that these results indicated very closely related species. There is a need for detailed morphological characterization to assign taxonomic identities to these taxa with certainty. Despite these limitations, our molecular data did yield some interesting results—including identifying new species not previously recorded in South Africa (*A. coolgardiensis, A. murrayana*), and confirming two species that were noted in planting records but for which taxonomic verification was lacking (*A. neriifolia, A. salicina*).

In conclusion, it is clear that available inventories of even supposedly well-known taxa can be misleading. A few representatives of this taxon is widespread and well known, there are however many species that will not be known except to a taxonomic expert. Better quantification of current introduction status is crucial for producing effective management strategies and for estimating the resources I needed control targeted populations of alien plants (Wilson et al. 2013). They are also essential if we are to have confidence in comparative analyses of invasions.

Acknowledgements

This work was supported by the South African National Department of Environment Affairs through funding of the South African National Biodiversity Institute's Invasive Species Programme and the DST-NRF Centre of Excellence for Invasion Biology. DMR acknowledges additional support from the National Research Foundation (grant 85417). I thank Sthembiso Gumede, Owethu Nomnganga, Virgil Jacobs, Jan-Hendrik Keet, Ulrike Irlich, George Sekonya, and various Working for Water teams for assistance in the field; Fiona Impson and Philip Weyl for alerting us about naturalised populations; and the Armstrong family for allowing us access to Damara Farm.

APPENDIX 2.1: A categorisation scheme for populations in the Unified Framework for Biological Invasions adapted for use in this study (Source: Blackburn et al. 2011).

Category	**Definition**
A	Not transported beyond limits of native range
B1	Individuals transported beyond limits of the native range, and in captivity or quarantine (i.e. individuals provided with conditions suitable for them, but explicit measures of containment are in place
B2	Individuals transported beyond limits of native range, and in cultivation (i.e. individuals provided with conditions suitable for them, but explicit measures to prevent dispersal are limited at best
B3	Individuals transported beyond limits of the native range, and directly released into novel environment
C0	Individuals released outside of captivity or cultivation in location here introduced, but incapable of surviving for a significant period

C1	Individuals surviving outside of captivity or cultivation in location where introduced, no reproduction
C2	Individuals surviving outside of captivity or cultivation at location where introduced. Reproduction occurring, but population is not self-sustaining
C3	Individuals surviving outside of captivity or cultivation in location where introduced. Reproduction occurring, and population is self-sustaining
D1	Self-sustaining population outside of captivity or cultivation, with individuals surviving a significant distance from the original point of introduction
D2	Self-sustaining population outside of captivity or cultivation, with individuals surviving and reproducing a significant distance from the original point of introduction
E	Fully invasive species, with individual dispersing, surviving and reproducing at multiple sites across a greater or lesser spectrum of habitats and extent of occurrence

APPENDIX 2.2: Species status report for *Acacia adunca* (using standardized metrics proposed by Wilson et al. 2014).

Species: *Acacia adunca* (Fabaceae)

Location: Groot Drakenstein (Bien Donne Farm), Western Cape. South Africa

Status: Naturalized; C3 under Blackburn Unified Framework for Biological Invasions; Individuals surviving in the wild in location where introduced, reproduction occurring, and population self-sustaining.

Potential: Large proportion of the country is suitable.

Abundance: ~1000 plants (2014); many seeds stored in the seedbank

Population Growth Rate: Not known.

Extent: One population covering area of 0.27 ha as a closed canopy (i.e. condensed canopy area is also0.27 ha).

Spread: From its native range, the seeds are spread by animal (ants and birds).

Impact: Has a potential to out-compete indigenous plants. *Acacia adunca* would fail a pre-border assessment as it scores higher than the threshold value of 6 that indicates species as being potentially invasive.

Threat: Not specifically studied, but likely similar to other Australian acacias (see Le Maître et al. 2011; Divers Distrib 17: 1015–1029).

Survey method(s) used: Systematic walked transects to generate point distributions. Herbarium specimens and the spotter website, South African Invasive Species, ISpot were examined, site delimitation found few plants outside the area.

Notes: Eradication plan in place. but based on the observed seedling recruitment events occurred after rain, it is believed that water may be the cause of population growth rate

Contact: invasivespecies@sanbi.org.za

Information compiled by: Nkoliso Magona, nkoliso@sun.ac.za

A2.3: Species status report for *Acacia cultriformis* (using standardized metrics proposed by Wilson et al. 2014).

Species: *Acacia cultriformis* (Fabaceae)

Location: Grahamstown (Makana Botanical Garden and Grey Dam), Eastern Cape.

Status: Naturalized; C3: Individuals surviving in the wild in location where introduced, reproduction occurring, and population self-sustaining.

Potential: Large proportion of the country is suitable.

Abundance: 35 plants (2015).

Population Growth Rate: No seedlings were found during the survey, so nothing is known of population growth rates.

Extent: Two populations covering area of 1.281 ha. (Condensed area of 0.052 ha).

Spread: In South Africa the species might be spread via seeds by people who are jogging or cycling.

Impact: Has a potential to out-compete indigenous plants. *Acacia cultriformis* would fail a pre-border assessment as it scores higher than the threshold value of 6 that indicates species as being potentially invasive.

Threat: Not specifically studied, but likely similar to other Australian acacias (see Le Maitre et al. 2011).

Survey method(s) used: Systematic walked transects to generate point distributions. Herbarium specimens and the spotter website, South African Invasive Species, ISpot were examined, site delimitation found few plants outside the area.

Notes: Eradication plan in place.

Contact: nkoliso@sun.ac.za; invasivespecies@sanbi.org.za

Information compiled by: Nkoliso Magona, nkoliso@sun.ac.za

A2.4: Species status report for *Acacia fimbriata* (using standardized metrics proposed by Wilson et al. 2014).

Species: *Acacia fimbriata* (Fabaceae)

Location: Grahamstown, South Africa

Status: Naturalized; D2 Self-sustaining population outside of captivity or cultivation, with individuals surviving and reproducing a significant distance from the original point of introduction.

Potential: Large proportion of the country is suitable.

Abundance: ~5 000 plants (2014); lots of seeds stored in the seedbank.

Population Growth Rate: Not known,

Extent: Three populations covering area of 53 ha. (Condensed area 0.73 ha)

Spread: From its native range the seeds are spread by animal (ants and birds). In South Africa the species may have dispersed via dumped garden waste from the introduced range. It was introduced to botanical garden and now it is found naturalized on a waste dumping site.

Impact: Has a potential to out-compete indigenous plants. *Acacia fimbriata* would fail a pre-border assessment as it scores higher than the threshold value of 6 that indicates species as being potentially invasive.

Threat: Not quantified.

Survey method(s) used: Systematic walked transects to generate point distributions. Herbarium specimens and the spotter website, South African Invasive Species, ISpot were examined, site delimitation found few plants outside the area.

Notes: Eradication plan in place. Based on the observed large levels of seedling recruitment events occurred after fire and water availability, it is believed that heat and water stimulate germination

Contact: invasivespecies@sanbi.org.za

Information compiled by: Nkoliso Magona, Stellenbosch University / SANBI

A2.5: Species status report for *Acacia piligera* (using standardized metrics proposed by Wilson et al., 2014).

Species: *Acacia piligera* (Fabaceae)

Location: Tokai, Western Cape

Status: Naturalized; C3 under Blackburn; Individuals surviving in the wild in location where introduced, reproduction occurring, and population self-sustaining.

Potential: Not quantified.

Abundance: ~174 plants (2015); lot of seeds stored on the seedbank.

Population Growth Rate: Not known.

Extent: One population covering area of 0.095 ha. (Condensed area of 0.095 ha).

Spread: In its native range, the seeds are dispersed by animal (ants). In South Africa it has not spread from its original cultivation area.

Impact: Not quantified

Threat: Not specifically studied, but likely similar to other Australian acacias (see Le Maitre et al. 2011).

Survey method(s) used: Systematic walked transects to generate point distributions. Herbarium specimens and the spotter website, South African Invasive Species, ISpot were examined, site delimitation found few plants outside the area.

Notes: Eradication plan in place. Seedling recruitment events occur particularly after rain and fire.

Contact: invasivespecies@sanbi.org.za

Information compiled by: Nkoliso Magona, nkoliso@sun.ac.za

A2.6: Species status report for *Acacia retinodes* (using standardized metrics proposed by Wilson et al., 2014).

Species: *Acacia retinodes* (Fabaceae)

Location: Tokai Arboretum, Western Cape

Status: Naturalized; C3; Individuals surviving in the wild in location where introduced, reproduction occurring, and population self-sustaining.

Potential: A large proportion of the country is suitable for this species.

Abundance: <~50 plants (2014); Relatively small seedbanks.

Population Growth Rate: Not known.

Extent: One population covering area of 0.267 ha. (Condensed area 0.251 ha)

Spread: In its native range, seeds are dispersed by animals (ants and birds).

Impact: Has the potential to out-compete indigenous plants. *Acacia retinodes* would fail a pre-border assessment as it scores higher than the threshold value of 6 that indicates species as being potentially invasive.

Threat: Not specifically studied, but likely similar to other Australian acacias (see Le Maitre et al. 2011; Divers Distrib 17: 1015–1029).

Survey method(s) used: Systematic walked transects to generate point distributions. Pamphlets were circulated to land owners; herbarium specimens and the spotter website, South African Invasive Species, ISpot were examined, site delimitation found few plants outside the area.

Notes: Eradication plan in place.

Contact: invasivespecies@sanbi.org.za

Information compiled by: Nkoliso Magona

A2.76: Species status report for *Acacia viscidula* (using standardized metrics proposed by Wilson et al., 2014).

Species: *Acacia viscidula* (Fabaceae)

Location: Newlands forest, Western Cape.

Status: Naturalized; C3; Individuals surviving in the wild in location where introduced, reproduction occurring, and population self-sustaining.

Potential: Large proportion of the country is suitable

Abundance: ~1200 plants (2014); vegetative reproduction?

Population Growth Rate: Not known.

Extent: Two populations covering area of 3.5 ha. (Condensed area of 0.077 ha).

Spread: From its native range, the seeds are spread by animal (ants and birds).

Impact: Has the potential to out-compete indigenous plants. *Acacia viscidula* would fail a pre-border assessment as it scores higher than the threshold value of 6 that indicates species as being potentially invasive.

Threat: Not specifically studied, but likely similar to other Australian acacias (see Le Maitre et al., 2011).

Survey method(s) used: Systematic walked transects to generate point distributions. Herbarium specimens and the spotter website, South African Invasive Species, ISpot were examined, site delimitation found few plants outside the area.

Notes: Eradication plan in place. It is a vigorous resprouter

Contact: invasivespecies@sanbi.org.za

Information compiled by: Nkoliso Magona, nkoliso@sun.ac.za

Chapter 3: Assessing the feasibility of eradication for naturalized Australia *Acacia* species in South Africa

This chapter is intended for submission to a journal.

Author contributions:

Nkoliso Magona, David M Richardson & John R Wilson: Planned the study

Nkoliso Magona: Collected data, did all statistical analyses and wrote the first draft of the paper

David M Richardson & John R Wilson: Edited the manuscript

Suzaan Kritzinger-Klopper: assisted with field work

Kanyisa Jama: provided field data for the year 2014 for the *A. fimbriata* population

Philip Weyl: initially discovered the *A. fimbriata* population and helped with initial field work

John R Wilson: Provided guidance on statistical analyses

The chapter is formatted in the style of *Biological Invasions* for standardization with the previous chapter.

Abstract

Attempting eradication for species occurring at low density is very important as it grants an opportunity to avoid impacts that could potentially result from a widespread alien plant invasion. However, to achieve eradication, target species must be well studied, and there must be adequate resources to conduct follow-up surveys. It is vital to find other naturalised species before they spread and become problematic to control. The aims of this study were to: (1) survey and map all naturalized populations of *Acacia adunca, A. cultriformis, A. fimbriata, A. piligera* (putative name), *A. retinodes* and *A. viscidula* in South Africa; (2) assess the invasion risk of the species across all sites; (3) assess the seed biology of the species (as this is known to be the factor most limiting to eradication in this group); and (4) assess the feasibility of eradicating these species. Detailed surveys and Australian weed-risk assessments were conducted. Seed viability and seed germination was conducted using tetrazolium solution and six treatments (control; smoke water; heat at 60^{0}C; heat at 100^{0}C;

heat at 60^{0}C and smoke water; and heat at 100^{0}C and smoke water). Post-fire surveys were conducted for three species to assess levels of recruitment from the seed bank.

The estimates of the seed bank size were *A. adunca* (~ 720000 seeds) *A. cultriformis* (51429), *A. fimbriata* (14090909), *A. piligera* (6324675) *A. retinodes* (99740) and *A. viscidula* (558442). Seed viability was very high for all species, for *A. adunca* (96%), *A. fimbriata* (90%) and *A. piligera* (92%). The germination was high (>50%) in 100^{0}C and 100^{0}C & smoke treatment. For *A. fimbriata* and *A. piligera* GLM showed that all the treatments are statistical significant *($p<0.05$)* from the control except for the smoke treatment *($p>0.05$)*. These results are in agreement with other studies that fire triggers the germination of *Acacia* species. However, for *A. adunca* statistical significant difference *($p<0.05$)* was observed at high temperature (100^{0}C) and smoke treatment only. All six species would have failed a pre-border risk assessment. All of these species can reach reproductive maturity by the following flowering season except for *A. retinodes* and three of these species produce large seedbanks. There was a significant reduction in the seed bank post fire for all the species.

Eradication is feasible for all of these targeted species except for *A. cultriformis* as there is a high chance that this species is distributed throughout South Africa in gardens. Annual clearing and surveys are recommended to prevent proliferation of infestations.

Keywords: Australian acacias; biological invasions eradication invasive species; management plan, tree invasions

3.1. Introduction

Biological invasions have increased exponentially worldwide in recent decades (Pyšek & Richardson, 2010; Latombe et al. 2017). Invasive species are an important component of human-caused environmental change (Richardson et al. 2011). Too often, management efforts are initiated when an alien species is already invasive and has spread over large areas, at which stage management is expensive and often ineffective (van Wilgen et al. 2011; van Wilgen and Richardson 2012).

A range of management approaches and tactics have been established to counteract the spread and the invasiveness of alien species (Wilson et al. 2011). For naturalized species that occur at only a few sites, eradication is a desirable management goal because there are substantial ecological and economic benefits when invading species are eliminated (Panetta 2007; Wilson et al. 2011; Moore et al. 2011).

Eradication is the elimination of every single individual of a species from an area to which recolonization is unlikely to occur (Myers et al. 1998). This is often set as a management goal that, if achieved, will reduce negative, and potential ecological impacts to the environment (Gherardi and Angiolini 2009; Panetta 2007; Mack and Lonsdale 2002). In assessing invasiveness and the feasibility of eradicating alien plants, it is crucial to understand key aspects of the biology and population dynamics of the species. This makes it possible to identify the risk posed by the species and ensures accurate planning for management.

Eradication of plant species can be time consuming and expensive (Rejmánek and Pitcairn 2002; Panetta 2007; Wilson et al. 2011). Eradication is sometimes not an appropriate goal for management, and many resources have been wasted on chasing eradication in situations where this was clearly a nonviable option. For, example, Rejmánek and Pitcairn (2002) summarized insights from many eradication attempts (they used a data set on exotic weed eradication attempts by the California Department of Food and Agriculture), where they explored whether the extent of the invasion matters in the eradication feasibility.

However, they found that eradication was often successful when applied to populations of <1 ha in extent. However, only a third of attempts to eradicate populations extending over 1–100 ha were successful, and only 25% of attempts were successful where the size of invasive populations was between 100 and 1000 ha. This shows that although eradication is often the preferred strategy in the management of new weed invasions, the conditions under which eradication can be achieved are very limited.

There are two stages of weed eradication (Panetta 2007): (1) the active phase that involves the control of established plants and new recruits; and (2) the phase where there is no recruitment but there is still a possibility of the plants being present due to the existence of the soil seedbanks. Hence, proactive management and long term monitoring is the key to the successful control of alien species. Furthermore, early detection, advanced search protocols (Panetta, 2007; Kaplan et al. 2012; Jacobs et al. 2014, Wilson et al. 2014) and regular visits to the targeted sites are crucial for achieving eradication, especially if the infestation is less than 100 ha in extent (Rejmánek and Pitcairn 2002). However, this could be because the field of invasion biology is poorly understood and has only recently gained attention. For eradication projects to be successful, the targeted species must be well studied and the project must be started before substantial spread has taken place (Wilson et al. 2011; Simberloff 2003; Panetta 2007). Adequate resources need to be ensured before the project starts, to allow for post-removal surveys, and during control, there should be regular follow-ups (Simberloff, 2009). Rouget et al. (2016) found that many wattle species have not reached their full invasiveness yet, and that several species introduced a long time ago are only starting to become invasive. They are yet to cause substantial impacts on biodiversity and ecosystem services.

This raises concerns, specifically for Australian acacias reproductive traits.

For an example, their ability to form large seedbanks which can stay dormant for up to 50-100 years (Gibson et al. 2011) and their capacity for long-distance dispersal are critical drivers in the progression along the introduction-naturalization-invasive continuum (Richardson and Kluge 2008). Thus, determining seed viability and the size of seedbanks is essential for assessing the feasibility of eradication. Hence, finding out the reproductive traits of naturalized acacias with limited distribution will be able to tell whether the species has a potential to become invasive or not, because invasive species, like Australian wattles, share some traits of invasiveness Gibson et al. (2011).

However, the time to visit a site before the targeted plants reach reproductive maturity is unknown at the beginning of the eradication programme, as the information about the length of pre-reproductive phase is not known. Regardless of whether the time of reproduction is known from the native range of a species, which does not guarantee that it will be the same in the introduced range. For example, Panetta (2007) conducted a study on *Mimosa pigra* L. invasions in central Queensland, Australia, site visits were scheduled at 4-month intervals, based on the information gained from the Northern Territory, where time from emergence to flowering was 180 days. However, in central Queensland, plants flowered as early as 67 days after germination. Hence, it is very important to study the reproductive biology of alien species in the introduced range.

The aims of this study are to: (1) Delimit populations of *Acacia adunca*, *A. cultriformis*, *A. fimbriata*, *A. piligera*, *A. retinodes* and *A. viscidula* in South Africa; (2) assess the invasion risk and the potential impacts of the species across all sites; (3) determine the size and viability of the current seedbank for each species and the triggers for germination and; (4) based on 1–3 to assess the feasibility of eradicating these species from South Africa.

3.2. Methods

3.2.1. Study species

There are six naturalized Australian acacias in South Africa that have not been studied in detail with records dating back as far as 50 years (For the extent and the size of these populations, see Chapter 2). This (2017) is the 6th year since the project has been active and management involved search and destroy strategy, with initial efforts carried out by SANBI (Wilson et al. 2013), and with project taking over from them using the same protocol they were using. The data collected (about the size and the distribution during the initial surveys) were also used in this thesis and the search and destroy methodology used, was also used in this study. For further details about management history, see Table 3.2. About the information for each of the six naturalized species, (S3.1) and photos of these species in Fig. 3.1.

A) B) C) D)

E)  F) 

Figure 3.1.A) *Acacia adunca;* B) *A. cultriformis*; C) *A. piligera*; D) *A. fimbriata*; E) *A. piligera* seeds; F) *A. retinodes*; G) *A. viscidula;* H) *A. adunca* bi-pinnate seedling.

All of these above-mentioned populations are climatically suitable to their current locations in South Africa (Motloung et al. 2014). Currently, only two species (*A. adunca* and *A. fimbriata*) from this study are listed under the NEM: BA Alien and Invasive Species Regulations (National Environmental Management: Biodiversity Act) as invasive (Department of Environmental affairs. 2016). They are listed as category 1a meaning that they need compulsory control (i.e. they are targets for nation-wide eradication).

3.2.2. Study sites

To find information on Australian acacias I reviewed the literature and looked for unpublished records, herbarium and museum records, and records in the Southern African Plant Invaders Atlas (SAPIA; Henderson and Wilson. (2017)), records on I-Spot (http://www.ispot.org.za/), and consulted the National Herbarium Computerized Information System (PRECIS online database http://posa.sanbi.org/intro_precis.php; Morris & Glen, 1978). Researchers working with *Acacia* species as well as botanical gardens in South Africa with specimens or collections of Australian *Acacia* species were also consulted regarding localities of naturalized wattle populations. I identified six naturalized populations, three of which occur in Cape Town, one in Newlands forest, two in Tokai, one at Bien Donne farm and two occur in Grahamstown (Eastern Cape), see Fig. 3.2. For further details, see Chapter 2.

3.2.3. Population Survey

To understand the extent of naturalization, spread and abundance of the target species, systematic surveys were carried out on all known populations and in areas surrounding these localities. Surveys were conducted along parallel transects 4 m apart. For each plant: plant canopy, height, stem basal diameter, presence or absence of reproductive structures and GPS coordinates were recorded.

Figure 3.2. Map of all sites with naturalized Australian acacias in South Africa.

The survey continued for up to 50m from the most isolated individual to assess dispersal (Zenni et al. 2009). Each plant was either hand-pulled or cut at the base and the stem applied with herbicide (Garlon 480 EC) to prevent sprouting or suckering as per the Working for Water (WfW) standard operational protocol (Zenni et al. 2009). Follow up search and destroy surveys were conducted seasonally during 2014-15 and twice a year during 2016 and 2017 to look for recruitment from the seedbank and suckering from the treated stumps. The management history including, number of populations, their localities, and number of individuals treated were recorded.

Table 3.1. Variables recorded during field surveys at different sites for assessing species invasive status and detection.

Date:						Site:							
Field-work team:						Species:							
Waypoint	GPS #	latitude decimal (S33.98...)	longitude decimal (E19.78...)	Canopy width 1 (cm)	Canopy width 2 (cm)	Height (cm)	Basal width 2 x (cm)	Buds (y/n)	Flowers (y/n)	Pods (y/n)	Seeds (y/n)	Resprout (y/n)	Notes

The GPS coordinates of all plants located were exported into ArcGIS10.4. Separate shape files were created for each species and distribution maps were produced. A composite map of all species was also created. The generated maps are indicative of the spatial distribution and extent of invasion of the *Acacia* species and will serve as a baseline for future invasion monitoring.

3.2.4. What is the invasion risk and impact potential?

A weed risk assessment was performed for six naturalized species, following the Australian Weeds Risk Assessment (A-WRA) protocol (Pheloung et al. 1999). The A-WRA was initially designed to be used as a pre-border assessment; however, it has also proved useful for species already in a region and as such has already been successfully applied in many parts of the world (see Kumschick & Richardson 2013). The A-WRA protocol uses 49 questions based on the biogeography, undesirable attributes, biology and ecology of the species. Guidelines on how to apply these assessments to areas outside Australia were used in this study (Gordon et al. 2010). Documented evidence from the literature and species data collected during the surveys were used for answering the questions in the A-WRA protocol. Answers to the questions were scored individually to provide a total score for the species, which in turn were used to indicate the risk of the species becoming invasive (Pheloung et al. 1999). Scores higher than six indicate that a species has a high risk of becoming invasive.

3.2.5. Seedbank dynamics and germination triggers

To estimate the total seed population, a square grid (25m x 25m) covering the densest part of the population was set up for *A. adunca*, *A. fimbriata, A. piligera* and *A. viscidula*. The grid was split into 5 x 5 m cells, and a soil sample was collected using a cylindrical soil corer (15cm deep and 7 cm in diameter) in each cell (giving 25 samples per grid). Morris (1997) reported that most seeds of wattles occur in the upper part of the soil seedbank. For *A. cultriformis* and *A. retinodes* no grid was created because the number of individuals was very low occupying a very small area with one or two scattered individuals, so I sampled under the canopy of big individuals in order to get an idea of the seedbank, and 6 samples of each were taken. Each soil core sample was emptied into a labelled brown paper bag and taken to the lab for analysis. In the lab, the soil samples were dried at 60˚C for 24 hours and sieved through a combination of 1mm, 2mm sieves and the seeds were counted. The estimate of the total seedbank for each population was calculated using the following formula: $S = 25 \,.\, n / (\pi.r^2).\, (p_{all} / p_{grid})$, where S is the number of seeds in the population; n is the number of seeds retrieved from each soil core sample; r is the radius of the soil corer (in m); 25 is used as the samples were taken over the 25 m^{-2} grid, and so gives an indication of the number of seeds in the grid; p_{grid} is the number of individual plants in the grid (during the first survey at the site); and p_{all} is the total number of plants recorded at the site including those in the grid (again during the first survey at the site). The part (pall / pgrid) gives a factor by which seed population in the grid must be multiplied to give total seed population. A core of 7cm in diameter samples 0.00385 m^2 of soil.

Due to insufficient seeds collected from the seedbank, I was able to conduct germination experiment for only three species *A. adunca, A. fimbriata* and *A. piligera* and smoke water and heat treatment at 100^0C experiment was not conducted for *A. adunca.* Five hundred seeds for (*A. adunca)* and six hundred seeds for (*A. fimbriata* and *A. piligera*) were used to explore the role of fire as a germination trigger using six treatments: i) smoke water treatment; ii) heat treatment at 100^0C; iii) heat treatment at 60^0C; iv) smoke water and heat treatment at 100^0C; v) smoke water and heat treatment at 60^0C; and vi) a control. For the smoke water treatment, replicates of 25 seeds were soaked in smoke water solution for 24 hours and then germinated in petri-dishes for 48 days to determine the seed germinability. For heat treatments, replicates of 25 seeds were heated in an oven for 10 minutes at 100˚C or 60˚C. For the combined treatment, smoke water was applied first and then the heat treatment.

All the petri-dishes were put into the growth chamber and 9ml average alternating day/night temperatures (10^0C during the night & 20 ^{0}C during the day) were set on a growth chamber. Artificial light were installed in a growth chamber and I buttons were used to monitor the light. The light was on 10/14 hour photoperiod, 10 hours the light will be on and then for 14 hours the seeds were exposed to the darkness. Every time the filter papers were changed 6ml of distilled water was added on a petri-dishes and 3ml drop of benomyl fungicide was added inside the petri dishes to prevent seeds from decaying Seed germinability for the different treatments were compared using a General Linear Model (GLM) with Poisson errors in R (R Core Team, 2017). The tetrazolium chloride test was used to assess seed viability (Peters, 2000). The seeds were scarified then soaked for 72h in a 1.0% 2, 3, 5-triphenyl tetrazolium chloride solution at room temperature in petri-dishes. Seed staining was evaluated as a surrogate for viability. Uniform staining is indicative of viability while fractional or lack of staining indicates non-viability.

3.2.6. Management and the eradication feasibility of these species nationwide.

All of these naturalized Australian acacias will require a management plan that includes estimation of time and costs to achieve eradication. To estimate the cost of the surveys, time taken, number of visits, and distance travelled were recorded. I used current rates for distance and car hire at the time obtained from Stellenbosch University car pool to estimate the cost. For remuneration rates for contract labour, I used standard Working for Water person-day estimations obtained from SANBI offices, Kirstenbosch.

I gathered information of these plants in their native and introduced ranges (spread of the population, reproductive abilities etc.) using Worldwide Wattle ver. 2. Available online at: www.worldwidewattle.com Accessed 15 August 2016; Poynton, 2009) Thereafter, I was able to make recommendations about strategies and management control based on population clearing, age at reproductive maturity, and detectability.

3.2.7. Post fire survey for *A. fimbriata, A. piligera*, and *A. retinodes*

The sites in Grahamstown and in Tokai were burnt in natural wild fires in August 2014 and March 2015 respectively. This gave me opportunity to unravel other aspects of the management of naturalized wattles such as seedbank depletion due to fire. I determined the effects of fire on the seedbank and the type of regeneration of the three species (*A. fimbriata, A. piligera* and *A. retinodes*. I surveyed the sites three months after the fire and every seedling was counted and uprooted to determine the type of regeneration. Soil samples were taken to determine the effect of fire on seedbank depletion using the method described in section 2.3.

3.3. Results

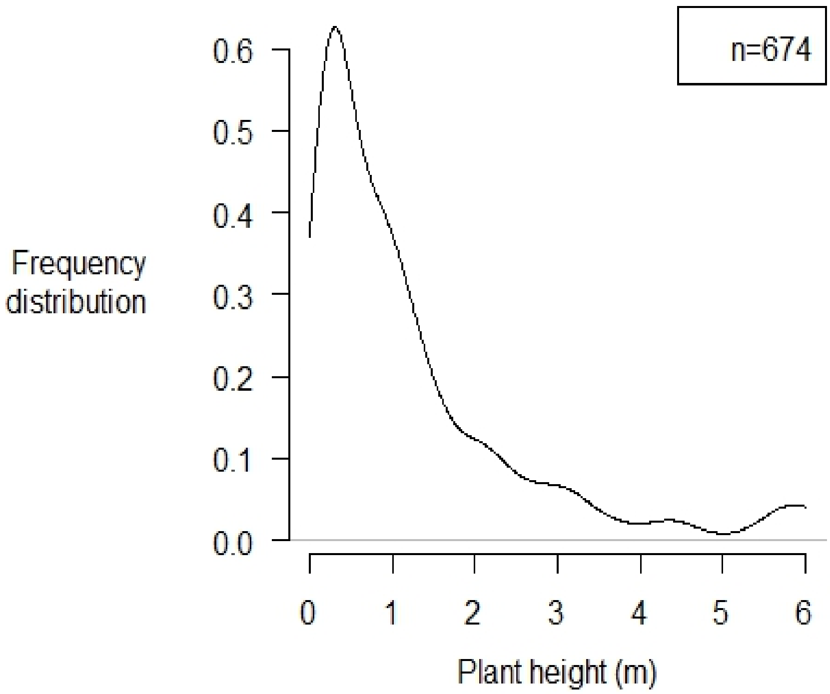
3.3.1. Current distribution and population dynamics

There was a similar trend in number of plants found with a few exceptions (Table 3. 2). *A. fimbriata, A. piligera, A. retinodes* and *A. viscidula* shows high number of seedling recruitment from the seedbank during 2014 and 2015. Whereas, *A. adunca* and *A. cultriformis* showed a decrease throughout the years (Table 3.2).

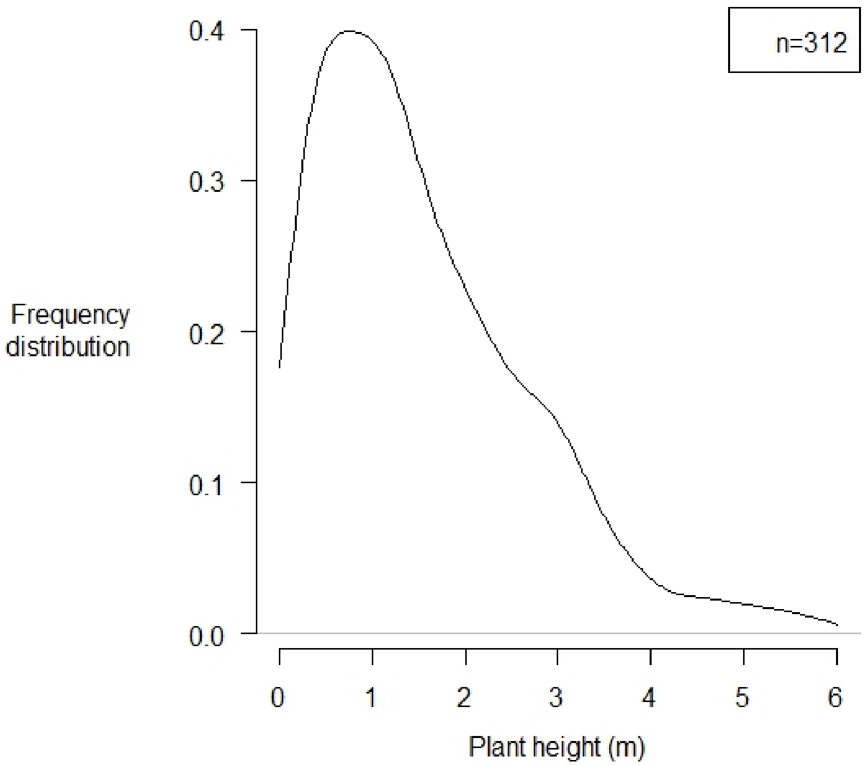
Table 3.2: Summary of the management time-line between "2011-2017" for the six naturalized species in South Africa. The numbers represent the individual plants that were either, pulled up or cut using a saw.

***Acacia* species**	**2011**	**2012**	**2014**	**2015**	**2016**	**2017**
A. adunca	122	na	1662	287	127	57
A. cultriformis	na	na	1	35	0	na
A. fimbriata	na	518	5512	5396	2569	na
A. piligera	na	na	na	11574	781	138
A. retinodes	na	120	43	340	225	150
A. viscidula	267	267	1490	730	150	230

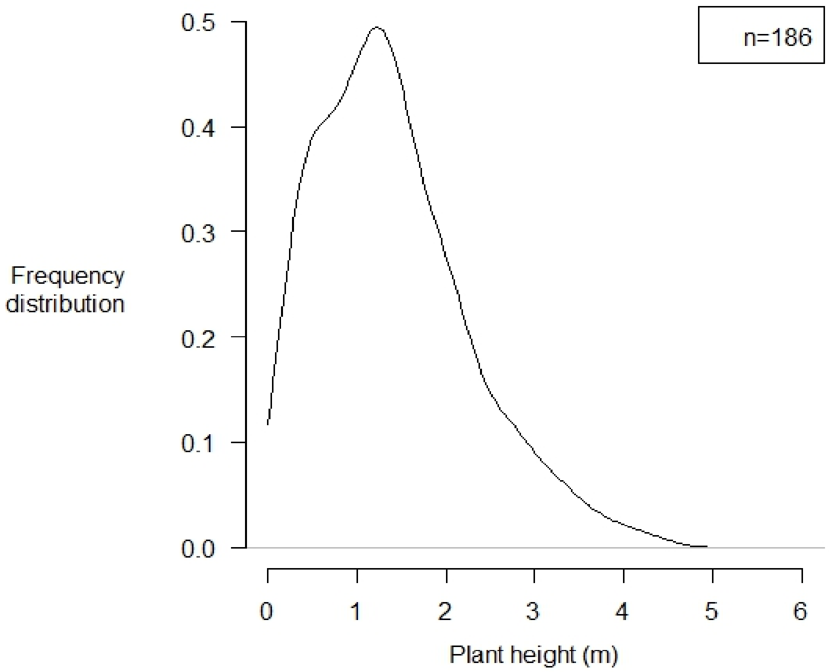
To track the history of populations and the whereabouts of the species, see Chapter 2. Total condensed area calculated using Arc GIS 10.4 (Wilson et al. 2014) for all species was less than 1 ha, although four species (*A. adunca, A. cultriformis, A. fimbriata, A. piligera*, and *A. viscidula* occur in more than 1 cluster, see Chapter 4, (Table 4.1). The size frequency distributions shown in Fig. 3.3 and the size at the onset of reproduction are shown in Fig. 3.4.


n=674
Frequency distribution
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
1
2
3
4
5
6
Plant height (m)

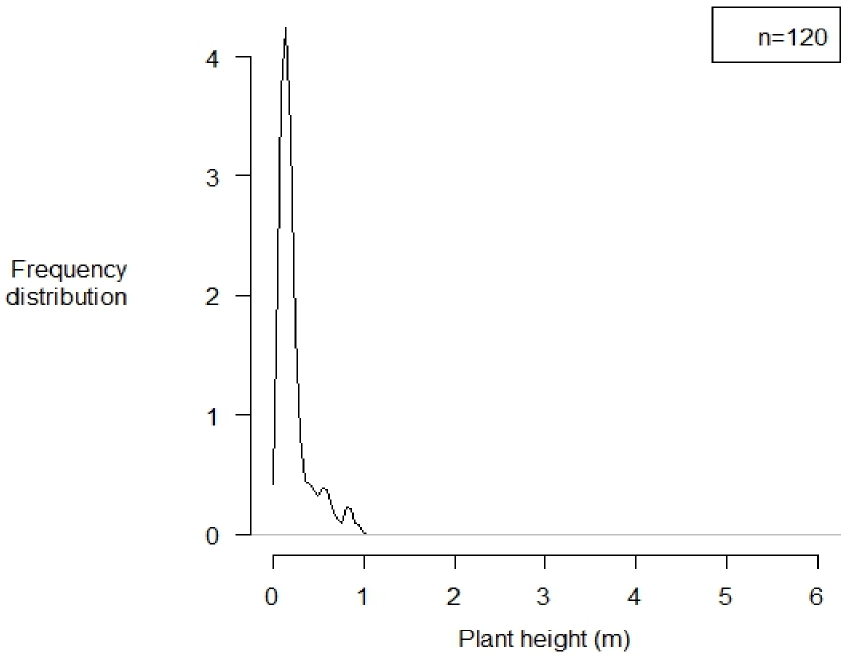
A)


n=312
Frequency distribution
0.4
0.3
0.2
0.1
0.0
0
1
2
3
4
5
6
Plant height (m)

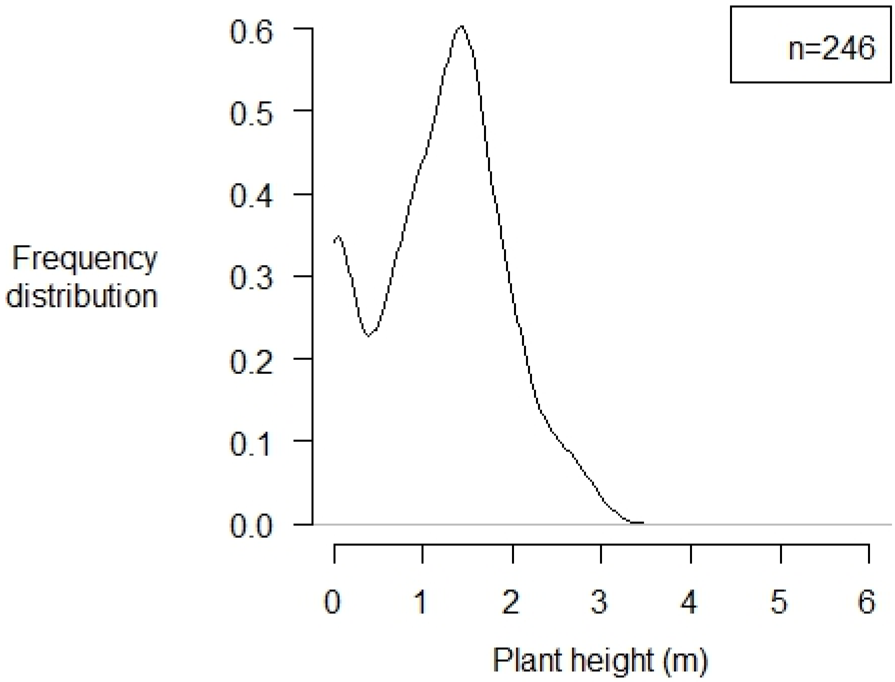
B)


n=186
0.5
0.4
0.3
0.2
0.1
0.0
Frequency distribution
0
1
2
3
4
5
6
Plant height (m)

C)

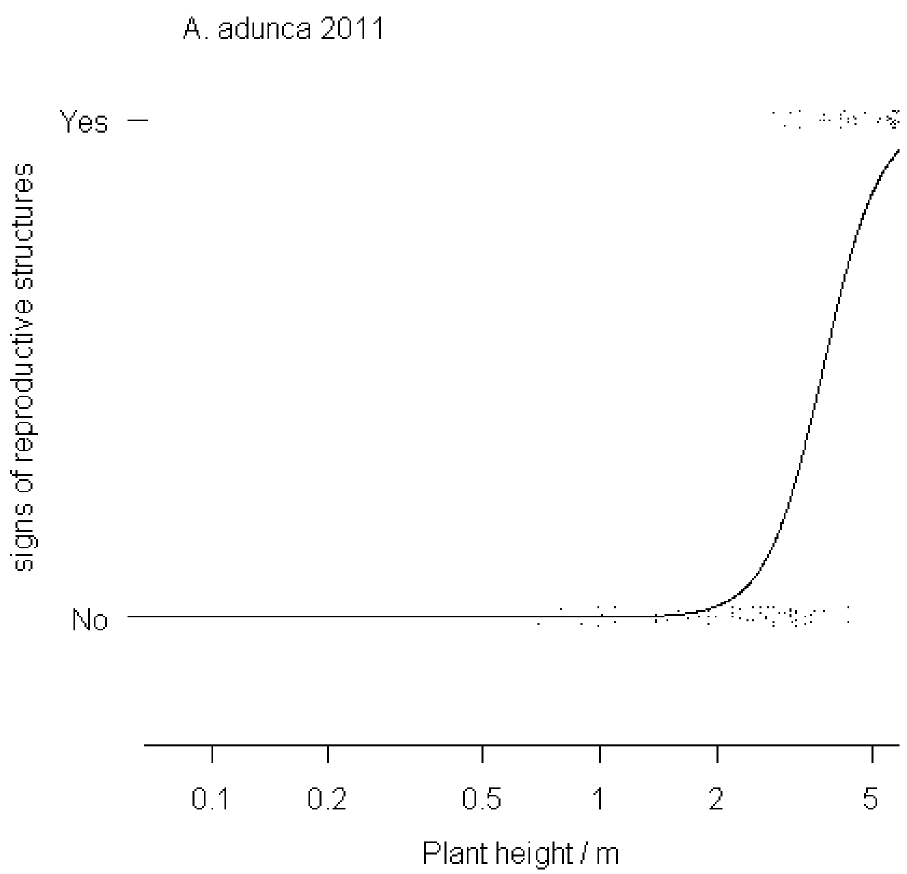

n=120
4
3
2
1
0
Frequency distribution
0
1
2
3
4
5
6
Plant height (m)

D)

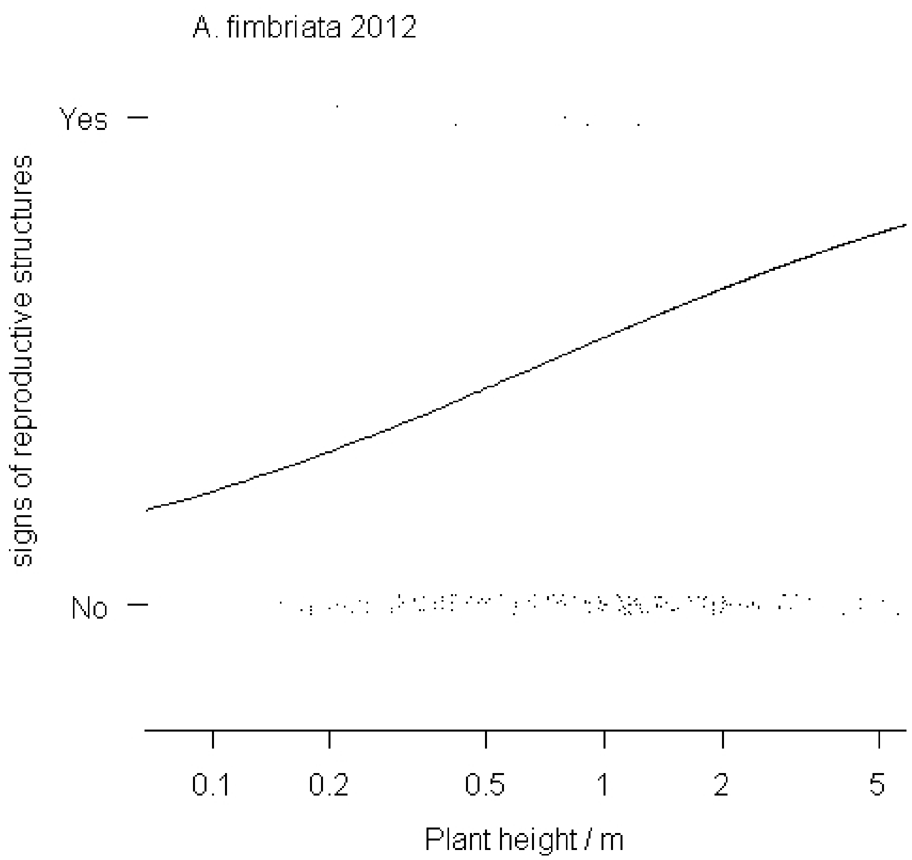


E)

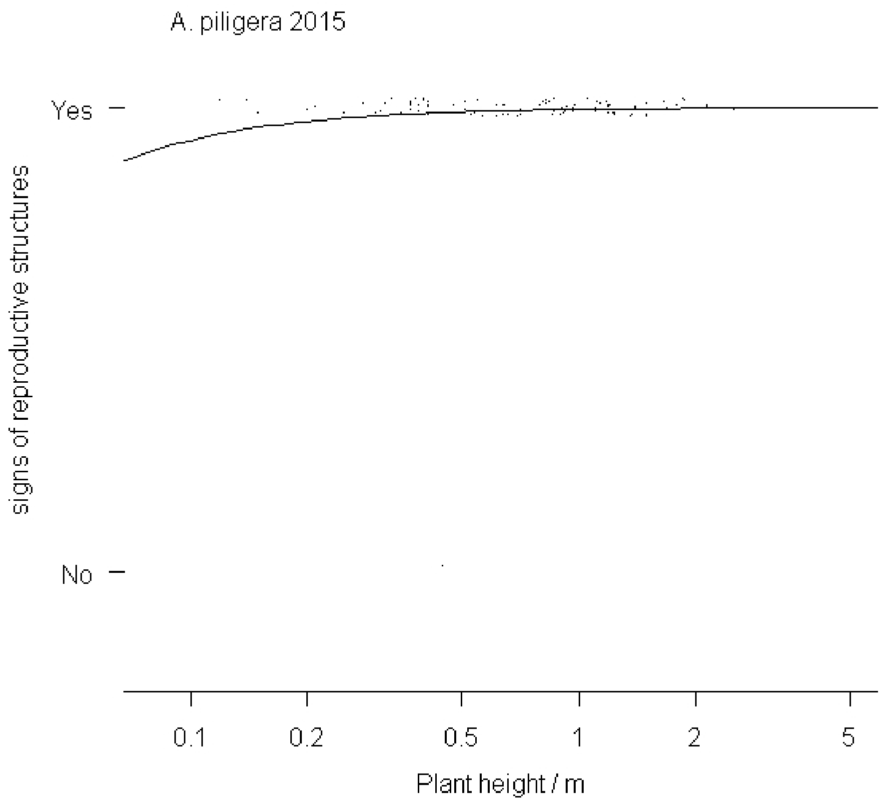
Figure 3.3. The plant height frequency distributions for A) *Acacia adunca*; B) *A. fimbriata*; C) *A. piligera*; D) *A. retinodes*; E) *A. viscidula.* The frequency distributions were produced using the function density [stats] in R.


A. adunca 2011
Yes
No
signs of reproductive structures
0.1
0.2
0.5
1
2
5
Plant height / m

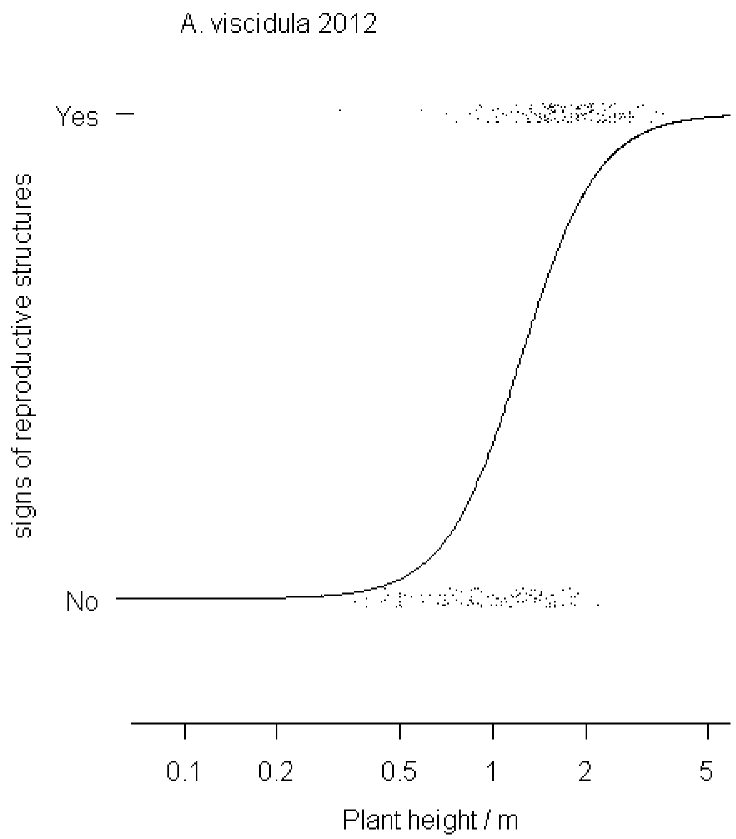
A)


A. fimbriata 2012
Yes
No
signs of reproductive structures
0.1
0.2
0.5
1
2
5
Plant height / m

B)


A. piligera 2015
Yes
No
signs of reproductive structures
0.1
0.2
0.5
1
2
5
Plant height / m

C)



D)

Figure 3.4. Size at reproduction (except for *A. retinodes* for which no flowers were recorded) for six naturalized Australian *Acacia* species in South Africa. A) *A. adunca*; B) *A. fimbriata*; C) *A. piligera*; D) *A. retinodes*; E) *A. viscidula*. The presence of seedpod stalks, seedpods or flowers were used as a proxy for reproductive maturity (jitter was added to prevent over plotting Geert et al. 2013). The fitted line for each site is from a generalized linear model with binomial errors and log (plant height) as the explanatory variable.

The dominant reproduction method for all species was from the seed, except for *A. viscidula*, which was from vegetative growth (suckering and resprout). Furthermore, I noticed that *A. viscidula* did not respond to the herbicide I applied, as I observed resprouting from treated stumps. A biological control agent *Dasineura dielsi* released against *A. cyclops* (Impson et al. 2011) and seed damage was noted on few *A. piligera* plants by an unknown insects.

3.3.2. Seedbank dynamics and germination triggers for *Acacia adunca, A. cultriformis A. fimbriata, A. piligera A. retinodes* and *A. viscidula*.

The average estimates of seedbank size for *A. adunca, A. cultriformis, A. fimbriata, A. piligera A. retinodes, A. viscidula* was significantly high, (Table 3.3). Seed viability was very high for all three tested species, *A. adunca*, (96%), *A. fimbriata* (90%) and *A. piligera* (92%).

Table 3.3. Records of the six naturalized *Acacia* species with the estimated seedbank size (*A. adunca*, *A. fimbriata, A. piligera* and *A. viscidula*); and six soil samples under the big different trees for (*A. cultriformis* and *A. retinodes*) of the population and the total invaded area.

***Acacia* species**	**Estimated seedbank size**	**Condensed canopy area**	**Area invaded (ha)**
A. adunca A. Cunn. ex G. Don	720000	0.27	0.27
A. cultriformis A. Cunn	51429	0.052	1.281
A. fimbriata A. Cunn. ex G. Don	14090909	0.73	53
A. piligera	6324675	0.095	0.095
A. retinodes Schlechtd.	99740	0.251	0.267
A. viscidula A. Cunn. ex Benth.	558442	0.077	3.45

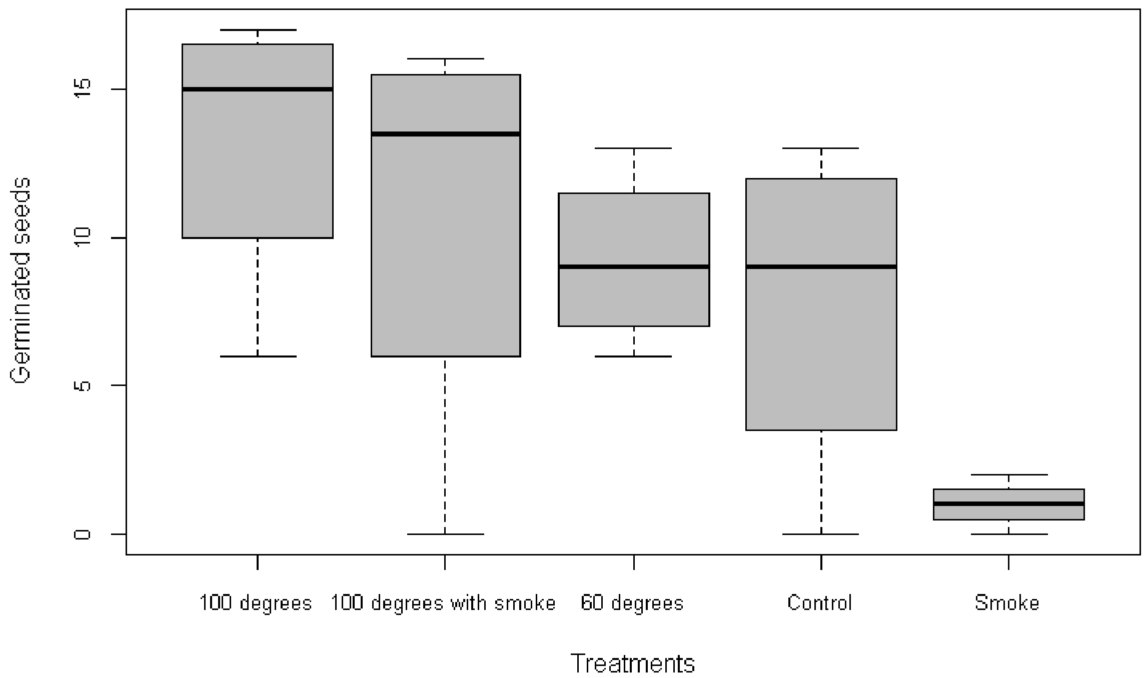
The germination percentage was high (>50%) in 100^{0}C and 100^{0}C & smoke treatment see Fig. 3.5. For *A. fimbriata* and *A. piligera* GLM showed that all the treatments are statistical significant *($p<0.05$)* from the control except for the smoke treatment *($p>0.05$)*. These results are in agreement with other studies that fire trigger the germination of *Acacia* species. However, for *A. adunca* statistical significant difference *($p<0.05$)* was observed at high temperature (100^{0}C) and smoke treatment only(Table 3.4). However, treatment with 100^{0}C for *A. adunca* resulted in the highest germination of 53%.

Table 3.4. Generalized linear model (glm1=glm(Gm~Treatment,data=data,family=poisson),

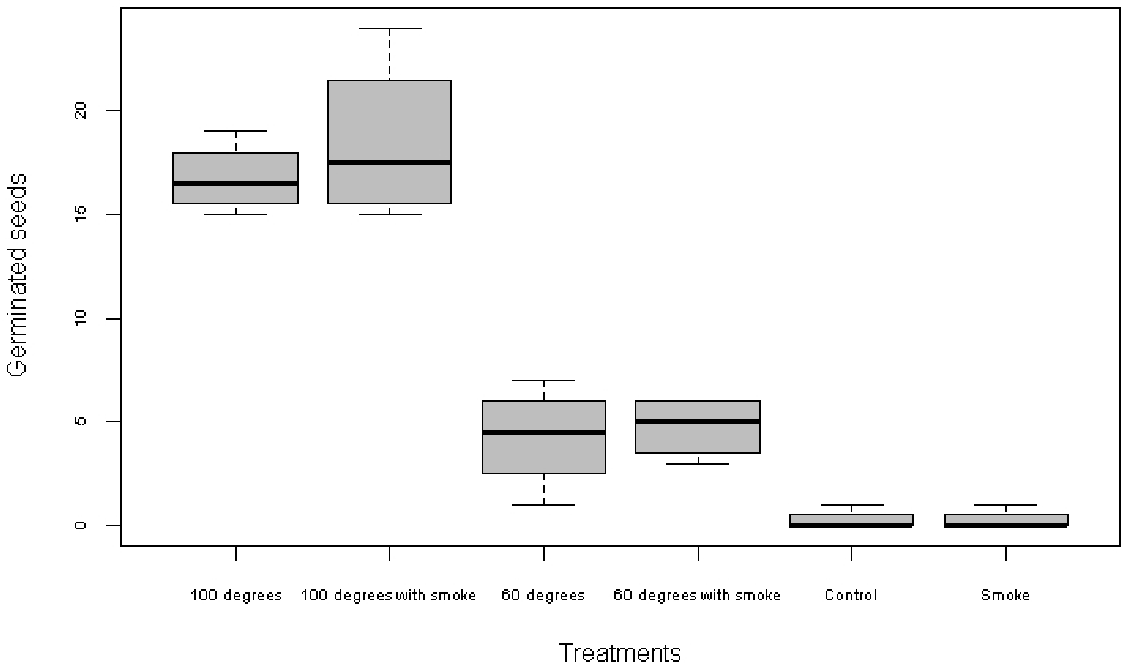
indicating influence of each treatment on germination of *Acacia* seeds. Values are differences from the control and statistically significance presented in bold and Significance is at P<0.05;. Details of the model see supplementary 3.2.

Treatment	*A. adunca*	*A. fimbriata*	*A. piligera*
(Intercept) Control	2e-16	0.166	0.165
100^{o}C	**0.018**	**2.99e-05**	**2.99e-05**
100^{o}C & smoke	0.165	**1.99e-05**	**1.99e-05**
60^{o}C & smoke	-	**0.006**	**0.006**
60^{o}C	0.467	**0.004**	**0.004**
Smoke	**0.000**	1.00	1.00

A) *A. adunca*


Germinated seeds
0
5
10
15
100 degrees
100 degrees with smoke
60 degrees
Control
Smoke
Treatments

B) *A. fimbriata*



C) *A. piligera*

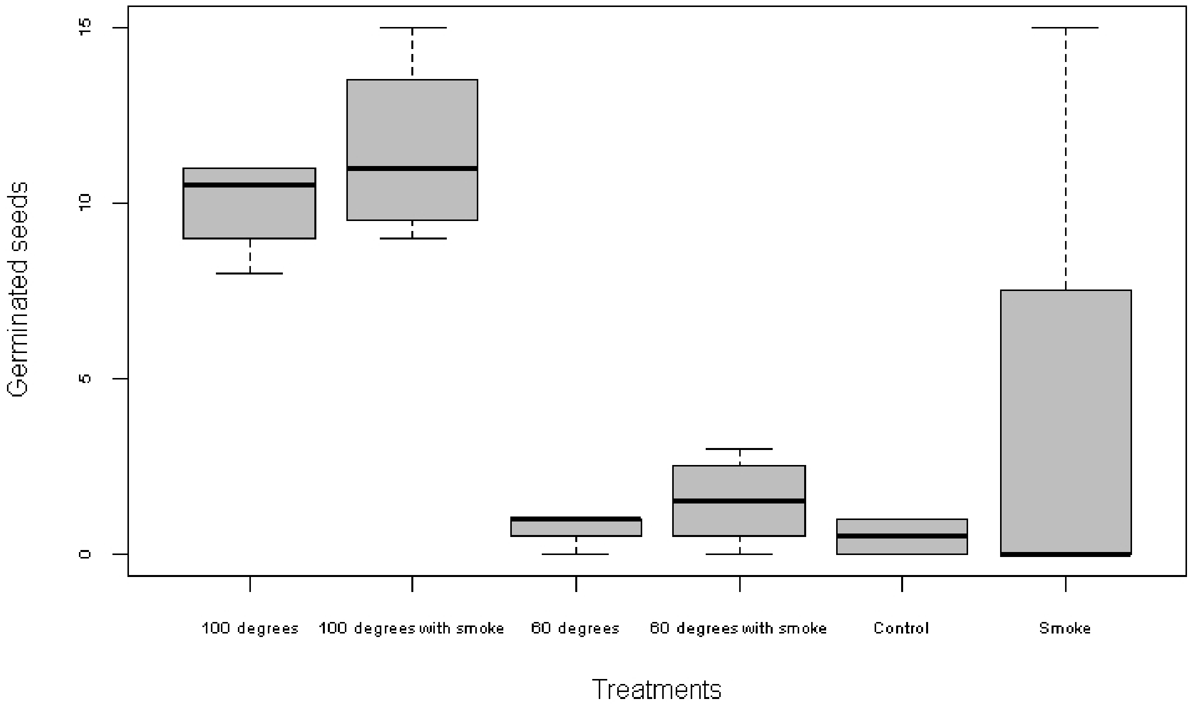


Fig. 3.5. Germinated seeds throughout the germination period under five treatments for A). *Acacia adunca*, and six pre-sowing treatments; B). *Acacia fimbriata*; C) *A. piligera* in the growing chamber.

3.3.3. What is the invasion risk and impact potential of *Acacia adunca, A. cultriformis A. fimbriata, A. piligera A. retinodes* and *A. viscidula*?

Based on the available literature and data collected in the field, Australian weed risk assessments were conducted on six species (see A3.1.). All of these species scored more than the cut-off of six and so would have failed a pre-border risk assessment (Pheloung 1999). In addition, based on the observations from the field, all six species pose a significant threat to the environment because of the large seed rain and copious amount of the seedlings coming up from the seedbank.

3.3.4. Post fire survey for *A. fimbriata, A. piligera, and A. retinodes.*

During our initial surveys after fire, I found a total of 5311, 8743 and 327 seedlings compared to previous number of 651, 186 and 16 for *A. fimbriata, A. piligera* and *A. retinodes* over an area of 0.73, 0.09 and 0.27 ha respectively. There were no resprouting plants detected and the seedbank was greatly reduced: down to 90% for *A. fimbriata*, 96% for *A. piligera* and 73% for *A. retinodes*.

3.3.5. Management and the eradication feasibility of these species nationwide.

Cost estimation for clearing these species were based on the Working for Water programme (WfW programme) guidelines per person day (Turpie et al. 2008). Previous clearing cost for these species was used to estimate the total amount needed per year, for at least ten years. All adult plants had been removed and the only concern now is the seeds in the seedbank. Thus, follow up surveys should be conducted once a year before the plants reach the reproductive maturity. However, re-surveys after fire maybe shortened to 6 months after fire especially during the rainy season as it was noticed that water encourages recruitment from the seedbank. Since all the sites are easily accessible and small in size, it would take two people for a maximum of four field days at a total cost of ~ ZAR 2645.9 per day for *A. fimbriata, A. piligera* and *A. viscidula*); two field days at a cost of ~ ZAR 1557.99 for (*A. adunca, A. cultriformis* and *A. retinodes*).The control for these plants is being is monitored by Stellenbosch University and South African National Biodiversity Institute, with cost during 2014-2016 for each species, (Table 3.5).

Table 3.5. Costs associated with conducting re-surveys of naturalized Australia *Acacia* species in South Africa between 2014 and 2016. Time spent searching and counting the plants. Number of localities that were visited and the cost of each trip are indicated.

Acacia species	Years		Time (hours)/day	No of people	No of visits/year		Total cost (ZAR)/year
A. adunca	2014-2016		9	2	4 (2014/15); 2 (2016)		5527.82
A. cultriformis	2014-2016		9	2	1 (2014/15);		500
A. fimbriata	2014-2016		9	2	4 (2014/15); 2 (2016)		59081.48
A. piligera	2015-2016		9	2	3 (2015/16);		7494.06
A. retinodes	2014-2016		9	2	4 (2014/15); 2 (2016)		2927.05
A. viscidula	2014-2016		9	2	4 (2014/5); 2 (2016)		9399.18

3.4.1. Discussion

Early detection and delimitation of the species targeted for eradication is very important especially if eradication is to be successful.. For example, Chapter 2 focussed on searching and mapping new population. As a result, there had been report of sightings of new population's *A. fimbriata* and *A. viscidula*, which occur as small clusters in close proximity to the source populations. There were also reports that *A. cultriformis* is common in gardens in Gauteng, Northern Cape and Western Cape, this however, is not surprising Poynton (2009) reported that this tree was among the most popular ornamental wattles in South Africa. This resulted to be possibly to assess eradication feasibility for the wattles.

One of the reasons that localized species have not increased their distribution is the size of introduction and unsuitable introduction site and the fear now is that, these species might start spreading. Human mediated disturbances such as hiking might contribute to the spread of these species especially five of these species occur near hiking trails (*A. fimbriata, A. cultriformis, A. piligera A. retinodes* and *A. viscidula*). Furthermore, in Tokai there are trucks that go in and out transporting wood and preparing them on site (in Tokai or in very close proximity to the sites). Motloung et al. (2014) has reported that more than half of South Africa is climatically suitable for acacia species. This suggests that many Australian acacias have the potential to become widespread invaders. It is clear that South Africa has quite a number of Australian trees occurring at low densities that have potential to become widespread and spread at a considerable distance from the parent plant (Chapter 2; Jacobs et al. 2015). One concerning issue about some of the Australian *Acacia* species is determining how they escape from introductory sites. For example, *Acacia fimbriata* had been mentioned in literature by various authors as a small population planted in Grahamstown Botanical Garden (Ross 1975; Poynton 2009; Van Wilgen et al. 2011), but now the population has naturalised across three sites. This suggests the possibility of more invasions of introduced species than previously recognised.

In the rest of this discussion I: i) elaborate on the current distribution of these species and how they might have escaped introductory sites; ii) discuss reproduction biology and invasion risks; and iii) outline management strategies.

3.4.2. Current distribution of naturalised Australian acacias in South Africa

The distribution of *A. adunca, A. piligera*, and *A. retinodes* are currently restricted to their introduced sites with high number of seedlings post rainy seasons and post fire. However, *A. fimbriata* and *A. viscidula* have spread a considerable distance (>100m) from their introduced sites. It is clear that these species have the ability to invade natural vegetation. This is of great concern, especially as a large area of South Africa is climatically suitable to these species (Motloung et al. 2014). In addition, the distribution data of these species suggest that *A. cultriformis* and *A. fimbriata* were the species most frequently planted throughout the country Poynton (2009) herbaria records (pers. Obs.). Most of the records did not have precise geographic localities, so it is likely that these species are present at other unknown sites (Chapter 2). Richardson et al. (2011), noted that frequently planted species have higher chances of invading. Given the difficulties predicting where horticultural plants have been introduced to (e.g. *A. cultriformis*), improving passive surveillance efforts will be important if an accurate estimate of nation-wide distribution is needed (e.g. through the distribution of flyers).

However, all of these species were introduced into either the botanical gardens, arboreta and forest station, thus, it would be ideal if additional active surveillance efforts focus on these places. There has been a record for these species dating back to several decades and they were part of forestry plantation (Poynton, 2009). The proximity of *A. fimbriata* and *A. viscidula* to hiking trails is worrying as it may lead to the accidental spread for these species. For example, during the resurvey for *A. piligera* in Tokai, I observed seedlings along the route to and from the population and I suspected that during our last visit, the seeds might have stuck on our shoes and they fell off as we were leaving the site. After noticing this trend of seeds re-growth along the trails, I checked seeds on the debris from the soles of the shoes prior to leaving the site and we did find seeds on the debris. Kaplan et al. (2014) found that road maintenance vehicles such as road graders and plantation harvesting vehicles or equipment spread *Acacia stricta* seeds. Hence, I became more conscious as I removed debris from our shoes before leaving the site as I also noticed that many seeds occurred on the leaf litter hence, I collected the leaf litter and removed the seeds in the laboratory. Based on this anecdotal evidence, clearing teams need to implement and practice this strategy for eradication attempts to be successful

3.4.3. Seedbank longevity and germination triggers of *Acacia* species

Understanding the seedbank ecology of Australian *Acacia* species is very important before the commencement of an eradication programme. Correia (2014) indicated that large stores of long-lived seeds with high levels of viability with high extent of the invasion might make it impossible to achieve eradication. Although initially seedbanks for *A. adunca, A. fimbriata, A. piligera* and *A. retinodes* were in proportion to those other invasive wattles (Richardson and Kluge 2008) in South Africa, there has been a significantly decrease in the extent of the seedbanks and the seedlings recruitment during the four years of managing these species. This could be attributed to various reasons, 1) The intensive fire that occurred in 2014 and 2015 for *A. fimbriata* and *A. piligera* stimulated thousands of seeds to germinate and also killing other seeds. Panetta (2007) indicated that disturbance such as fire; accelerate the depletion of the seedbank. 2) The availability of the rain have contributed to the seedling recruitment, as it was noted from this study that seedling recruitment was significantly high during rainy seasons.

For *A. cultriformis*, people that have this tree in their garden had never seen recruitment from the seedbank although the trees produced flowers and seeds. I assumed that this species produces sterile seeds or other organisms (like insects, birds, rodents or being destroyed by above/below ground micro-organisms) are consuming the seeds. For example, on another *Acacia* species (*A. piligera*), I noticed that hundreds of seeds found on the ground and on leaf litter were damaged or rotten and most of them had holes and there were insects that were discovered on the leaf litter. Richardson and Kluge (1998) mentioned that predation or rotting of the seeds were one of the reasons for the loss of the seeds on the leaf litter.

For *A. viscidula*, the dominant reproductive method is vegetative hence; there is very low recruitment from the seedbank and lower numbers of seeds found from the seedbank similar to *A. implexa* (Kaplan et al. 2012). It is clear that heat treatment together with smoke stimulates seed-germination, this corroborates findings/observations with previous studies (Donaldson et al. 2013b) that heat stimulates the germination of wattles.

3.4.4. Management

Seedbanks can hamper eradication efforts (Zamora et al. 1989). However, for all of the species assessed in this study, eradication as a management goal is feasible, as there is no longer input to the seedbank and these species are restricted in distribution. Nevertheless, for *A. fimbriata* there is high probability that there are still other populations in Grahamstown area that have not been discovered yet. During this study, I noted that *A. fimbriata* spread through the garden waste; hence, botanical garden managers need to be attentive about where they dispose garden waste as they could contribute to the spread of invasive species.

For all of these species except *A. viscidula*, they are not prone to resprouting; hence controlling these species is feasible. For A. viscidula, vegetative reproduction and small seedbank is beneficial to the management plan. There is no threat that this species will spread, and mechanical clearing, using pixel to dig up the root suckers is the only control that could work for this species.

Acknowledgements

This work was supported by the South African National Department of Environmental Affairs through its funding of the South African National Biodiversity Institute Invasive Species Programme, with support from the DST-NRF Centre of Excellence for Invasion Biology. I thank Sthembiso Gumede, Virgil Jacobs, Jan-Hendrik Keet, Owethu Nomnganga, Ulrike Irlich, Fiona Impson, George Sekonya, and various Working for Water teams for assistance in the field.

Appendix 3.1: Australian Weed Risk Assessment for naturalized Australian *Acacia* species in South Africa (*A. adunca, A. cultriformis, A. fimbriata, A. piligera, A. retinodes* & *A. viscidula*). "Ans." = Answer and "Yes[2]" – the squared number from yes refers to the reference used to get the results, negative points can be scored for certain questions.

	Acacia species											
	A. adunca		*A. cultriformis*		*A. fimbriata*		*A. piligera*		*A. retinodes*		*A. viscidula*	
Questions	**Ans.**	**Score**	**Ans.**	**Score**	***Ans.***	**Score**	**Ans.**	**Score**	**Ans.**	**Score**	**Ans.**	**Score**
Is the species highly domesticated?	No[1]	0	No[1]	0	No[1]	0	No[1]	0	No[1]	0	No[1]	0
Species suited to South African climates?	Yes[2]	2	Yes[2]	1	Yes[2]	1	Yes	2	Yes[2]	2	Yes[2]	1
Quality of climate match data (0-low; 1-intermediate; 2-high)	Yes[2]	2	Yes[2]	2	High	2	Yes	1	Yes[2]	2	Yes[2]	2
Broad climate suitability (environmental versatility)	Yes[2]	1	Yes[2]	2	Yes[2]	1	No[4]	0	No	0	No	0
Native or naturalized in regions with extended dry periods?	No[4]	1	Yes[5]	1	No	0	Yes[1]	1	No	0	No	0
Does the species have a history of repeated introductions outside its natural range?	No	0	No	0	No	0	No	0	No	0	No	0
Naturalized beyond native range	Yes[1]	2	Yes	2	Yes	2	Yes	2	Yes.	2	Yes	2
Garden/amenity/disturbance weed	Yes	1	Yes.	2	Yes	3	Yes[3]	2	Yes	1	Yes.	1
Weed of agriculture/horticulture/forestry	No	0	NA	?	NA	?	NA	?	NA	?	Yes	2

	Acacia species											
	A. adunca		*A. cultriformis*		*A. fimbriata*		*A. piligera*		*A. retinodes*		*A. viscidula*	
Questions	**Ans.**	**Score**	**Ans.**	**Score**	***Ans.***	**Score**	**Ans.**	**Score**	**Ans.**	**Score**	**Ans.**	**Score**
Environmental weed	NA	?	NA	?	NA	?	NA	?	NA	?	NA	?
Congeneric weed	Yes	2	Yes	2	Yes	2	Yes	2	Yes	2	Yes	2
Produces spines, thorns or burrs	No	0	No	0	No	0	No	0	No	0	No	0
Allelopathic	No	0	No	0	No	0	No	0	No	0	No	0
Parasitic	No	0	No	0	No	0	No	0	No	0	No	0
Unpalatable to grazing animals	NA	?	NA	?	NA	?	NA	?	NA	?	NA	?
Toxic to animals	No	0	No	0	No	0	NA	?	NA	?	NA	?
Host for recognised pests and pathogens	NA	?	NA	?	NA	?	NA	?	NA	?	NA	?
Causes allergies or is otherwise toxic to humans	NA	?	NA	?	NA	?	NA	?	NA	?	NA	?
Creates a fire hazard in natural ecosystems	NA	?	NA	?	NA	?	NA	?	NA	?	NA	?
Is a shade tolerant plant at some stage of its life cycle	No	0	No	0	No	0	Yes[4]	0	Yes[4]	0	Yes[4]	0
Grows on infertile soils	Yes	1	Yes	1	Yes	1	Yes	1	Yes	1	Yes	1
Climbing or smothering growth habit	No	0	No	0	No	0	No	0	No	0	NA	?
Forms dense thickets	Yes	1	No	0	Yes	1	No	0	No	0	Yes	1
Aquatic	No	0	No	0	No	0	No	0	No	0	No	0
Grass	No	0	No	0	No	0	No	0	No	0	No	0
Nitrogen fixing woody plant	Yes	1	Yes	1	Yes	1	Yes	1	Yes	1	Yes	1

	Acacia species											
	A. adunca		*A. cultriformis*		*A. fimbriata*		*A. piligera*		*A. retinodes*		*A. viscidula*	
Questions	**Ans.**	**Score**	**Ans.**	**Score**	***Ans.***	**Score**	**Ans.**	**Score**	**Ans.**	**Score**	**Ans.**	**Score**
Geophyte	No	0	No	0	No	0	No	0	No	0	No	0
Evidence of substantial reproductive failure in native habitat	No	0	No	0	No	0	No	0	No	0	No	0
Produces viable seed	Yes	1	No	0	Yes	1	Yes	1	Yes	1	Yes	1
Hybridises naturally	No.	1	NA	1	Yes[4]	1	NA	?	NA	?	No	1
Self-fertilisation	NA	?	NA	?	NA	?	NA	?	NA	?	NA	?
Requires specialist pollinators	No	0	No	0	No	0	No	0	No	0	NA	?
Reproduction by vegetative propagation	No.	-1	No.	-1	No.	-1	No.	-1	No.	-1	Yes	1
Minimum generative time (years)	1 year	1	1 year	1	1 year	1	1 year	1	1 year	1	1 year	1
Propagules likely to be dispersed unintentionally	Yes	1	Yes	1	Yes	1	Yes	1	Yes	1	Yes	-1
Propagules dispersed intentionally by people	No	-1	Yes	1	No	-1	No	-1	No	-1	No	1
Propagules likely to disperse as a produce contaminant	No	-1	Yes	1	Yes	1	NA	?	Yes	1	No	-1
Propagules adapted to wind dispersal	NA	?	NA	?	NA	?	NA	?	NA	?	NA	?
Propagules buoyant	NA	?	NA	?	NA	-1	NA	?	NA	?	NA	?
Propagules bird dispersed	NA	?	NA	?	NA	-1	NA	?	NA	?	NA	?
Propagules dispersed by other animals (externally)	NA	?	NA	?	NA	?	NA	?	NA	?	NA	?

	Acacia species											
	A. adunca		*A. cultriformis*		*A. fimbriata*		*A. piligera*		*A. retinodes*		*A. viscidula*	
Questions	**Ans.**	**Score**	**Ans.**	**Score**	***Ans.***	**Score**	**Ans.**	**Score**	**Ans.**	**Score**	**Ans.**	**Score**
Propagules dispersed by other animals (internally)	NA	?	NA	?	NA	?	NA	?	NA	?	NA	?
Prolific seed production	Yes	1	NA	?	Yes	1	Yes	1	Yes	1	Yes	?
Evidence that a persistent propagule bank is formed (>1 yr)	Yes	1	No	-1	Yes	1	Yes	1	Yes	1	Yes	1
Well controlled by herbicides	Yes	-1	Yes	1	Yes	1	Yes	1	Yes	1	No	1
Tolerates or benefits from mutilation, cultivation or fire	Yes	1	NA	?	NA	?	Yes	1	Yes	1	Yes	1
Effective natural enemies present in Australia	NA	?	NA	?	NA	?	NA	?	NA	?	NA	?
Total score	**17**		**16**		**18**		**17**		**17**		**19**	

- [1] This paper
- [2]Motloung et al. (2014).
- [3]Kodela & Harden (2002).
- [4]Worldwide Wattle ver. 2.
- [5]Poynton (2009)

Chapter 4: Conclusion and Management Recommendations

4.1 **General conclusion**

Australian acacias have been moved around the world by humans and they have become part of many ecosystems (Griffin et al. 2011). Almost a third of the world's surface area has climatic conditions that are similar to those in Australia (Richardson et al. 2011). This, together with on-going heavy propagule pressure, has contributed to the success of these species in their introduced ranges. Besides the widespread current invasions, this has also led to a very large invasion debt Rouget et al. (2016) in many parts of the world, where invasions have not had time to manifest.

The large literature on Australian acacias and the variety of interventions for dealing with invasive wattles in South Africa Kaplan et al. 2012; Carruthers et al. 2011; van Wilgen et al. 2011.; Le Roux et al. 2011 Poynton 2009) provide an opportunity to explore the population dynamics of wattles that have not become widespread yet. Such information will help to inform policy on how to manage them. This study has provided information that will be useful to decision makers on how to manage naturalized species. I hope that the methods applied in this study could also be used for other plant taxonomic groups.

Given the history of widespread *Acacia* species in South Africa, the findings of this study will help inform the control of these species.

Chapters 2 and 3 provided assessments of the current status of species with limited distribution and the management of naturalised species. I discuss some general conclusions from this work in the following sections.

4.2. Status of introduced *Acacia* species in South Africa

Chapter 2 looked at the number of introduced *Acacia* species based on literature, determined the status of these species by revisiting the sites where they were introduced or recorded, and confirmed the identity of these species using molecular approach. The results indicated that the number of introduced *Acacia* species was underestimated, as I found many more species than were previously recorded. This study resulted in 45 new records of species in South Africa to add to the 70 species previously known to have been introduced (Richardson et al. 2011). Based on observed populations, there are 17 invasive species—I have categorized 16 species as E and one as category D3, 8 naturalized species as category C3, and 25 species fall into category C1 (see appendix 2.1).

Some of these species (*A. aquaria, A. latipes, A. leptospermoides, A. saliciformis, A. ulicina,* and *A. uncifera*) have not been recorded outside Australia before (Richardson et al. 2011). There were two major reasons for this discrepancy. First, during the survey I noticed that some of the species planted in Damara Farm in the Western Cape were not in the list provided by Stellenbosch University's Department of Forestry and it is not clear where these seeds came from. Second, several species present in the National Herbarium in Pretoria were not on the previous lists. This was because the herbarium records had not yet been digitised.

Despite my best efforts, my survey probably missed some species, (in particular, as ornamental trees were often introduced without locality data). Despite this, I am confident that most of these species that I did not re-find from their previously recorded sites are no longer in South Africa or are only restricted to small ranges. Most of the introduction sites (experimental farms) have been converted to agricultural land and biological agents attack some of the remaining species with the result that there is no sign of reproduction. However, it was worrying to find a site with more than 20 previously undocumented *Acacia* species (Damara farm). There may be other localities like Damara Farm where multiple species have been cultivated and potentially still exist, because repeated forestry trials were done at several sites across the country and most of these plantings were subsequently left unmanaged (Poynton, 2009). There is a need to engage with old Department of Forestry officials who might have been involved in the planting of these plants, so that I can be able to track down other possible site similar to Damara Farm.

The second part of Chapter 2 looked at old planting records to check for errors (incorrect naming, misspelling etc.). Unlike other groups in which many taxa were found to be misidentified (e.g. *Melaleuca* spp.; Jacobs et al. 2017), I found relatively few errors in the historical records. This is probably because there has been a large body of research in South Africa on different aspects of Australian acacias or, alternatively, taxon is easier to identify than other groups.

I also used DNA barcoding to help to resolve species identity. However, it was surprising to find that this molecular approach could not resolve all taxonomic uncertainties. If a well-studied genus like *Acacia* lacks complete data on GenBank, the shortage of data is likely to be much worse for less well-studied groups. This casts doubt on the current value of DNA barcoding to contribute to detailed inventories of many plant groups.

4.3. Current distribution, potential impacts & the risk posed by naturalised species and their eradication feasibility.

There are six naturalized *Acacia* species, which, prior to this study, had not been assessed in detail. Four species were found to occur in the Western Cape (*A. adunca, A. piligera, A. retinodes* and *A. viscidula* with two populations at Newlands forest and Newlands residential area), and two species occur in Eastern Cape (*A. cultriformis* and *A. fimbriata*). These populations appear to be spatially restricted; they occur either in arboreta or botanical gardens (although *A. cultriformis, A. fimbriata* & *A. viscidula* have spread, they are in close proximity with the original source population, easily accessible, and hence easy to delimit).

Low propagule pressure and short residence times likely explain the current restricted ranges. However, given a chance to spread further, widespread invasion would likely result. For example, *A. fimbriata* was mentioned in the old literature as an established species in the Grahamstown Botanical Garden (Ross 1975). However, dispersal of the species through disposal of garden waste to a favourable environment allowed the species to flourish.

The introductory pathway for the abovementioned species is largely linked to forestry (Poynton 2009), although the attractiveness of some of these species means that they were also traded as ornamental plants (Poynton 2009; Donaldson et al. 2014a). For example, *A. cultriformis* has been distributed throughout South Africa as a garden or street plant.

All of these species scored more than 6 in the Australian Weed Risk Assessment (AWRA, Pheloung et al. 1999), which is a threshold value that indicates that the species have the potential of becoming invasive. This means that these species would fail a pre-border assessment. Besides the fact that all of these species have large potential ranges in South Africa based on climatic suitability (Motloung et al. 2014), they pose a threat to the indigenous biodiversity. However, unlike other invasive wattles in South Africa, these species have, so far, accumulated relatively small seedbanks.

4.4. **Management strategies for the naturalised wattles**

Based on the restricted current extent, high potential risk of invasiveness and low seedbank size for these species, eradication should remain as a management strategy for these species. Currently, only two species addressed in this study (*A. adunca* and *A. fimbriata*) are listed in the 2016 NEM: BA A&IS Regulations. Both are listed as a category 1a which means that they need compulsory control. I propose that of the remaining species, *A. piligera, A. retinodes* and *A. viscidula* should be listed as category 1a and that *A. cultriformis* should be listed as category 1b; the last-mentioned species occurs in gardens, which means that the extent of its distribution cannot easily be delimited. However, it has not been seen to become a widespread invader yet and it has not been seen to be producing viable seeds, so it is not a priority for management (see Table 4.1 for details).

Table: 4.1. Record of naturalized *Acacia* species, number of sites, location in South Africa, with 1a= meaning listed in NEMB:A .

Species	Number of sites	NEM:BA category	Proposed NEM:BA category	Sites	Landscape context	Population size
A adunca A. Cunn. ex G. Don	1	1a		Bien Donne' farm, Drakenstein, S33. 844071º E18. 98163º	Experimental farm	>100 trees
A cultriformis A. Cunn	2 (likely many more)		1b	Makana Botanical Gardens & Gray Dam, Grahamstown	Botanical garden, & adjacent to Gray dam	~20 trees
A fimbriata A. Cunn. ex G. Don	3	1a		Makana Botanical Garden S33.31806˚ E26.152862˚, adjacent to PJ Olivier High School & Gray dam. Grahamstown	Botanical garden, dumping site & adjacent to Gray dam	>200 trees
A retinodes Schlechtd.	1		1a	Tokai Arboretum, S34.06037 E18.41543	Adjacent to SANParks offices	>100 trees
A ulicifolia (Salisb.) Court var. brownei (Poir.) Pedlez	1		1a	Tokai Arboretum	Adjacent to SANParks offices	2 plants
A piligera	1		1a	Tokai Arboretum	Opposite parking area	>150 trees
A viscidula A. Cunn. ex Benth.	2		1a	Newlands forest and neighbouring suburbs S33.97545˚ E18.44396˚	Forest and in suburb setting	>150 trees

Annual search and destroy strategies have proved to be effective. 2017 is the 6th year of the project and the population size has been reduced. The recommendation from this study is that this management strategy should continue to achieve positive results. *A. viscidula* produced vigorous suckers and herbicide applications was not effective in preventing suckering. I recommend that secateurs, and pick saws should be used to cut and dig up root suckers. This method has been successful in reducing the density of *A. viscidula* individuals during follow-ups. For *A. fimbriata* I recommend that eradication is feasible but this species needs to be monitored and there is a need to raise awareness of the threat posed by this species. All these species are in active phase of eradication, as all established plants removed, but there is new recruitments from the seedbank every year.

Finally, this study showed the importance of intervening before invasions become widespread, as it is cost effective. However, knowing the extent of spread, potential risk and understanding the seed biology of targeted species for eradication feasibility are essential. This information helps to know the time to visit a site before the targeted plants reach reproductive maturity. I believe that this information is essential for effective management. Studying and understanding the reproductive biology of these species has provided insights on why these species are only starting to invade now.

Acacia fimbriata has been recently discovered in Grahamstown in 2011 naturalized at a dumping area. I believed that it was spread through garden waste from the source population that is Makana Botanical garden. This species has been recorded in Makana Botanical garden in the 1950s (Ross 1975) and only to be discovered a few decade ago naturalized on a new location. Most these species occurring at low densities were recently introduced two decades ago, it is also possible for them to be re-introduced through garden waste into climatically suitable environment and become widespread. In addition, These species are used as ornamental plants in their native range, if they could escape from their cultivated areas, a fear is that people might plant them in their gardens as they grow very fast and does not require much water. For *A. adunca*, this species occupies a small area but a year after the management of the species had commenced, another few trees were seen not far from the source population. There has been agricultural work that has been going on the site, which means some the seeds might have been spread through that process.

However, good news is that, many seedlings of this species do not survive as they suffer from the dry conditions and now there is very few people working on the farm and that might limit their accidental spread. On the other side, for *A. fimbriata* and *A. viscidula*, there is a big concern for these species as they have shown a tendency to spread, the number of people hiking near or on the sites where these species occur is increasing every day, and they might contribute to their spread. For *A. piligera* and *A. retinodes*, although there is high number of seedlings that still comes up due to fire, very small number of individual survive to reach reproductive stage and there is concerns that might contribute to their spread via the trucks that go in and out of Tokai to collect wood. However, my estimate of their seedbanks, suggest that the seedbanks have decreased drastically over time. I did not find that *A. cultriformis* produced fertile seeds hence it is not a big concern although it will be important to delimit this species in South Africa.

5. References

Adamson RS (1938) The vegetation of South Africa. The vegetation of South Africa

Blackburn, TM, Pyšek P, Bacher S, Carlton JT, Duncan RP, Jarošík V, Wilson JRU, Richardson DM (2011). A proposed unified framework for biological invasions. Trends Ecol Evol 26:333-339

Castro-Díez P, Godoy O, Saldaña A, Richardson DM (2011) Predicting invasiveness of Australian *Acacia* species on the basis of their native climatic affinities, life-history traits and human use. Divers Distrib 17:934-945

Carruthers J, Robin L, Hattingh JP, Kull CA, Rangan H, van Wilgen BW (2011) A native at home and abroad: the history, politics, ethics and aesthetics of acacias. Divers Distrib 17:810-821

Correia M, Castro S, Ferrero V, Chrysostom J, Rodríguez-Echeverría S (2014) Reproductive biology and success of invasive Australian acacias in Portugal. Bot J Linn Soc 174:574–588

Dickie IA, Bennett BM, Burrows, LE, Nuñez MA, Peltzer DA, Porté A, Richardson DM, Rejmánek M, Rundel PW, van Wilgen BW (2014) Conflicting values: ecosystem services and invasive tree management. Biol Invasions 16:705-719

Donaldson JE, Hui C, Richardson DM, Wilson JRU, Robertson MP, Webber BL (2014a) Invasion trajectory of alien trees: the role of introduction pathway and planting history. Global Change Biol 20:1527–1537

Donaldson JE, Richardson DM, Wilson JRU (2014b) The seed ecology of an ornamental wattle in South Africa—why has *Acacia elata* not invaded a greater area? S Afr J Bot 94:40–45

Flora of Australia (2001) Volume 11B, Mimosaceae, Acacia part 2. Melbourne: ABRS/CSIRO Publishing

Funk VA, Richardson KS (2002) Systematic data in biodiversity studies: use it or lose it. Syst Biol 51:303-316

Gaertner M, Breeÿen AD, Hui C, Richardson DM (2009) Impacts of alien plant invasions on species richness in Mediterranean-type ecosystems: a meta-analysis. Progr Phys Geogr 33:319–338

Garcia-Milagros E, Funk VA (2010) Improving the use of information from museum specimens: Using Google Earth© to geo-reference Guiana Shield specimens in the US National Herbarium. Front Biogeogr 2(3)

Gibson MR, Richardson DM, Marchante E, Marchante H, Rodger JG, Stone GN, Byrne M, Fuentes-Ramírez A, George N, Harris C, Johnson SD, Le Roux JJ, Miller JT, Murphy DJ, Pauw A, Prescott MN, Wandrag EM, Wilson JRU (2011) Reproductive biology of Australian *Acacia* species: Important mediator of invasiveness. Divers Distrib 17:911-933

Gordon DR, Onderdonk DA, Fox AM, Stocker RK (2008) Consistent accuracy of the Australian weed risk assessment system across varied geographies. Divers Distrib 14:234–242

Gordon DR, Riddle B, Pheloung PC, Ansari S, Buddenhagen C, Chimera C, Daehler CC, Dawson W, Denslow JS, Tshidada NJ, LA Rosa A, Nishida T, Onderdonk DA, Panetta FD, Pyšek P, Randall RP, Richardson DM, Virtue JG, Williams PA (2010) Guidance for addressing the Australian Weed Risk Assessment questions. Plant Prot Quart 25:56-74

Griffin AR, Midgley SJ, Bush D, Cunningham PJ, Rinaudo AT (2011) Global uses of Australian acacias. Recent trends and future prospects. Divers Distrib 17:837–847

Henderson L (1998) Southern African Plant Invaders Atlas (SAPIA). Appl Plant Sci12: 31–32

Henderson L, Wilson JRU (2017) Changes in the composition and distribution of alien plants in South Africa: an update from the Southern African Plant Invaders Atlas (SAPIA). Bothalia 47(2), a2172.https://doi.org/10.4102/abc.v47i2.2172

http://www.inaturalist.org/taxa/181887-*Acacia-cultriformis*. Accessed on 15 March 2016.

The University of California and Jepson herbaria, Berkeley. Accessed on 10 March 2016.http:// www.ucjeps.berkeley.edu/consortium/

Impson FAC, Kleinjan CA, Hoffmann J H, Post JA, Wood AR (2011) Biological control of Australian acacia species and *Paraserianthes lophantha* (Willd.) Nielsen (Mimosaceae) in South Africa. Afr Entomol 19:186–207

Jacobs LEO, Richardson DM, Wilson JRU (2014) *Melaleuca parvistaminea* Byrnes (Myrtaceae) in South Africa: invasion risk and feasibility of eradication. S Afr J Bot 94:24–32

Jacobs LEO, van Wyk E, Wilson JRU (2015) Recent discovery of small-naturalized populations of *Melaleuca quinquenervia* (Cav.) ST Blake in South Africa. Biol Invasions Rec 4: 53–59

Jacobs LEO, Richardson DM, Lepschi B, Wilson JRU (2017) Quantifying errors and omissions in the listing of alien species: Melaleuca in South Africa as a case study. Neobiota 32:89-105

Kaplan H, van Niekerk A, Le Roux, JJ, Richardson, DM, Wilson JRU (2014) Incorporating risk mapping at multiple spatial scales into eradication management plans. Biol Invasions 16:691-703

Kaplan H, van Zyl HWF, Le Roux JJ, Richardson DM, Wilson JRU (2012) Distribution and management of *Acacia implexa* (Benth.) in South Africa: A suitable target for eradication? S Afr J Bot 83: 23-35

Klein H (2011) A catalogue of the insects, mites and pathogens that have been used or rejected, or are under consideration, for the biological control of invasive alien plants in South Africa. Afr Entom 19:515-549

Klock MM, Barrett LG, Thrall PH, Harms KE (2016) Differential plant invasiveness is not always driven by host promiscuity with bacterial symbionts. AoB Plants 8: plw060

Kodela Harden, Flora of NSW Vol. 2 (2002) Conn BJ Tame TM Austr Syst Bot 9:827-857

Kotzé I, Beukes H, Van den Berg E, Newby T (2010) National invasive alien plant survey. Agricultural Research Council, Institute for Soil, Climate and Water, Report No. GW/A/2010/21.

Kull CA, Shackleton CM, Cunningham PJ, Ducatillon C, Dufour-Dror, JM, Esler KJ, Friday JB, Gouveia AC, Griffin AR, Marchante E, Midgley SJ, Pauchard A, Rangan H, Richardson DM, Rinaudo T, Tassin J, Urgenson LS, von Maltitz GP, Zenni RD, Zylstra MJ (2011) Adoption, use and perception of Australian acacia species around the world. Divers Distrib 17:822-836

Kull CA, Rangan H (2008) *Acacia* exchanges: wattles, thorn trees, and the study of plant movements. Geoforum 39:1258-1272

Kull CA, Tassin J (2012) Australian *Acacia* species: useful and (sometimes) weedy. Biol Invasions 14: 2229-2233

Kumschick S, Richardson DM (2013) Species-based risk assessments for biological invasions: Advances and challenges. Divers Distrib 19:1095-1105

Latombe G, Pyšek P, Jeschke JM, Blackburn TM, Bacher S, Capinha C, Costello MJ, Fernández M, Gregory RD, Hobern D, Hui C, Jetz W, Kumschick S, McGrannachan C, Pergl J, Roy HE, Scalera R, Squires ZE, Wilson JRU, Winter M, Genovesi P, McGeoch MA (2017) A vision for global monitoring of biological invasions. Biol Conserv 213:295–308

Le Maitre DC, Gaertner M, Marchante E, Ens EJ, Holmes PM, Pauchard A, O'Farrell PJ, Rogers AM, Blanchard R, Blignaut J , Richardson DM (2011) Impacts of Australian *Acacia* species on ecosystem services and functions, and options for restoration. Divers Distrib 17:1015–1029

Le Maitre DC, van Wilgen B, Gelderblom C, Bailey C, Chapman R, Nel J (2002) Invasive alien trees and water resources in South Africa: case studies of the costs and benefits of management. For Ecol Manage 160:143-159

Le Maitre DC, Versfeld DB, Chapman RA (2000) The impact of invading alien plants on surface water resources in South Africa: Preliminary assessment. Water SA 26:397-408

Le Roux JJ, Brown G K, Byrne M, Ndlovu J, Richardson DM, Thompson GD, Wilson JRU (2011) Phylogeographic consequences of different introduction histories of invasive Australian acacia species and *Paraserianthes lophantha* (Fabaceae) in South Africa. Divers Distrib 17:861-871

Mack RN, Lonsdale WM (2002) Eradicating invasive plants: hard-won lessons for islands. In: Veitch CR, Clout MN (eds). Turning the tide: the eradication of invasive species. IUCN, Gland, Switzerland and Cambridge, UK: IUCN SSC Invasive Species Specialist Group. 164–172

Marais C, van Wilgen BW, Stevens D (2004) The clearing of invasive alien plants in South Africa: a preliminary assessment of costs and progress. S Afr J Sci 100:97-103

McGeoch MA, Spear D, Kleynhans EJ, Marais E (2012) Uncertainty in invasive alien species listing. Ecol Appl 22:959-971

Morris J, Glen H (1978) PRECIS, the National Herbarium of South Africa (PRE) Computerized information system. Taxon 27:449-462

Motloung RF, Robertson MP, Rouget M, Wilson JRU (2014) Forestry trial data can be used to evaluate climate based species distribution models in predicting tree invasions. NeoBiota 20:31–48

Moore JL, Runge MC, Webber BL, Wilson JRU (2011) Contain or eradicate? Optimising the goal of managing Australian acacia invasions in the face of uncertainty. Divers Distrib 17:1047–1059

Myers JH, Savoie A, van Randen E (1998) Eradication and pest management. Ann Rev Entomol 43:471-491

Panetta F, Csurhes S, Markula A, Hannan-Jones M (2011) Predicting the cost of eradication for 41, Class 1 declared weeds in Queensland. Plant Prot Quart 26:42

Parmentier I, Duminil J, Kuzmina M, Philippe M, Thomas DW, Kenfack D, Chuyong GB, Cruaud C, Hardy OJ (2013) How effective are DNA barcodes in the identification of African rainforest trees? PLoS ONE 8(4), p.e54921

Pheloung PC, Williams PA, Halloy SR (1999) A weed risk assessment model for use as a biosecurity tool evaluating plant introductions. J Environm Manage 57:239-251

Poynton RJ (2009) Tree Planting in Southern Africa: Other Genera. Department of Forestry Pretoria, South Africa, 773pp

Pyšek P, Richardson DM (2010) Invasive species, environmental change and management, and ecosystem health. Ann Rev Environm Res 35:25-55

Regan HM, Colyvan M, Burgman MA (2002) A taxonomy and treatment of uncertainty for ecology and conservation biology Ecol Appl 12:618–628

Rejmánek M, Pitcairn M J (2002) When is eradication of exotic pest plants a realistic goal? In: C. R. Veitch and M. N. Clout (eds) Turning the tide: the eradication of invasive species. pp. 249-253, IUCN Invasive Species Specialist Group, Gland

Rejmánek M, Richardson DM (2013) Trees and shrubs as invasive alien species – 2013 update of the global database. Divers Distrib 19:1093-1094

Richardson DM, Carruthers J, Hui C, Impson FAC, Miller J, Robertson MP, Rouget M, Le Roux JJ, Wilson JRU (2011) Human-mediated introductions of Australian acacias—a global experiment in biogeography. Divers Distrib 17:771–787

Richardson DM, Kluge RL (2008) Seed banks of invasive Australian acacia species in South Africa: role in invasiveness and options for management. Perspect Plant Ecol Evol Syst 10:161–177

Richardson DM, Le Roux JJ, Wilson JRU (2015) Australian acacias as invasive species: lessons to be learnt from regions with long planting histories. Sth Forests 77:31–39

Richardson DM, Pyšek P, Rejmánek M, Barbour MG, Panetta FD, West CJ (2000) Naturalization and invasion of alien plants: concepts and definitions. Diversity and Distributions 6: 93–107

Richardson DM, Rejmánek M (2011) Trees and shrubs as invasive alien species – a global review. Divers Distrib 17:788-809

Ross JH (1975) The naturalized and cultivated exotic *Acacia* species in South Africa. Bothalia 11:463-470

Rouget M, Richardson DM, Nel JL, Le Maitre DC, Egoh B, Mgidi T (2004) Mapping the potential spread of major plant invaders in South Africa using climatic suitability. Divers Distrib 10:475-484

Rouget M, Robertson MP, Wilson JRU, Hui C, Essl F, Renteria JL, Richardson DM (2016) Invasion debt–quantifying future biological invasions. Divers Distrib 22:445–456

Simberloff D (2003) How much information on population biology is needed to manage introduced species? Conserv Biol 17:83-92

Simberloff D (2009). The role of propagule pressure in biological invasions. Annu. Rev. Ecol. Evol. Syst 40:81–102

Stoeckle MY, Gamble CC, Kirpekar R, Young G, Ahmed S, Little DP (2011) Commercial teas highlight plant DNA barcode identification successes and obstacles. Sci Rep 1.42 doi:10.1038/srep00042

Strydom M, Esler KJ, Wood AR (2011) *Acacia saligna* seed banks: Sampling methods and dynamics, Western Cape, South Africa. S Afr J Bot 79:140–147

Strydom M, Veldtman R, Ngwenya MZ, Esler KJ (2017) Invasive Australian *Acacia* seed banks: Size and relationship with stem diameter in the presence of gall-forming biological control agents. PLOS ONE 12(8): e0181763 https://doi.org/10.1371/journal.pone.0181763

Van Wilgen BW, Carruthers J, Cowling RM, Esler KJ, Forsyth AT, Gaertner M, Hoffman MT, Kruger FJ, Midgley GF, Palmer G, Pence G, Raimondo DC, Richardson DM, van Wilgen NJ, Wilson JRU (2016) Ecological research and conservation management in the Cape Floristic Region between 1945 and 2015: History, current understanding and future challenges. Trans Royal Soc S Afr 71:207-303 http://dx.doi.org/10.1080/0035919X.2016.1225607

Van Wilgen BW, Dyer C, Hoffmann JH, Ivey P, Le Maitre DC, Richardson DM, Rouget M, Wannenburgh A, Wilson JRU (2011) A strategic approach to the integrated management of Australian *Acacia* species in South Africa. Divers Distrib17:1060–1075

Van Wilgen BW, Richardson DM, (2014) Challenges and trade-offs in the management of invasive alien trees. Biol Invasions 16:721-734

Visser V Langdon B, Pauchard A, Richardson DM (2014) Unlocking the potential of Google Earth as a tool in invasion science. Biol Invasions 16:513–534

Wilson JRU, Caplat P, Dickie IA, Hui C, Maxwell BD, Nuñez MA, Pauchard A, Rejmánek M, Richardson DM, Robertson MP, Spear D, Webber BL, van Wilgen BW, Zenni RD (2014) A standardized set of metrics to assess and monitor tree invasions. Biol Invasions 16:535-551

Wilson JRU, Gairifo C, Gibson MR, Arianoutsou M, Bakar BB, Baret S, Celesti-Grapow L, Ditomaso JM, Dufour-Dror JM, Kueffer C, Kull CA, Hoffmann JH, Impson FAC, Loope LL, Marchante E, Marchante H, Moore JL, Murphy DJ, Tassin J, Witt A, Zenni RD, Richardson DM (2011) Risk assessment, eradication, and biological control: Global efforts to limit Australian acacia invasions. Divers Distrib 17:1030-1046

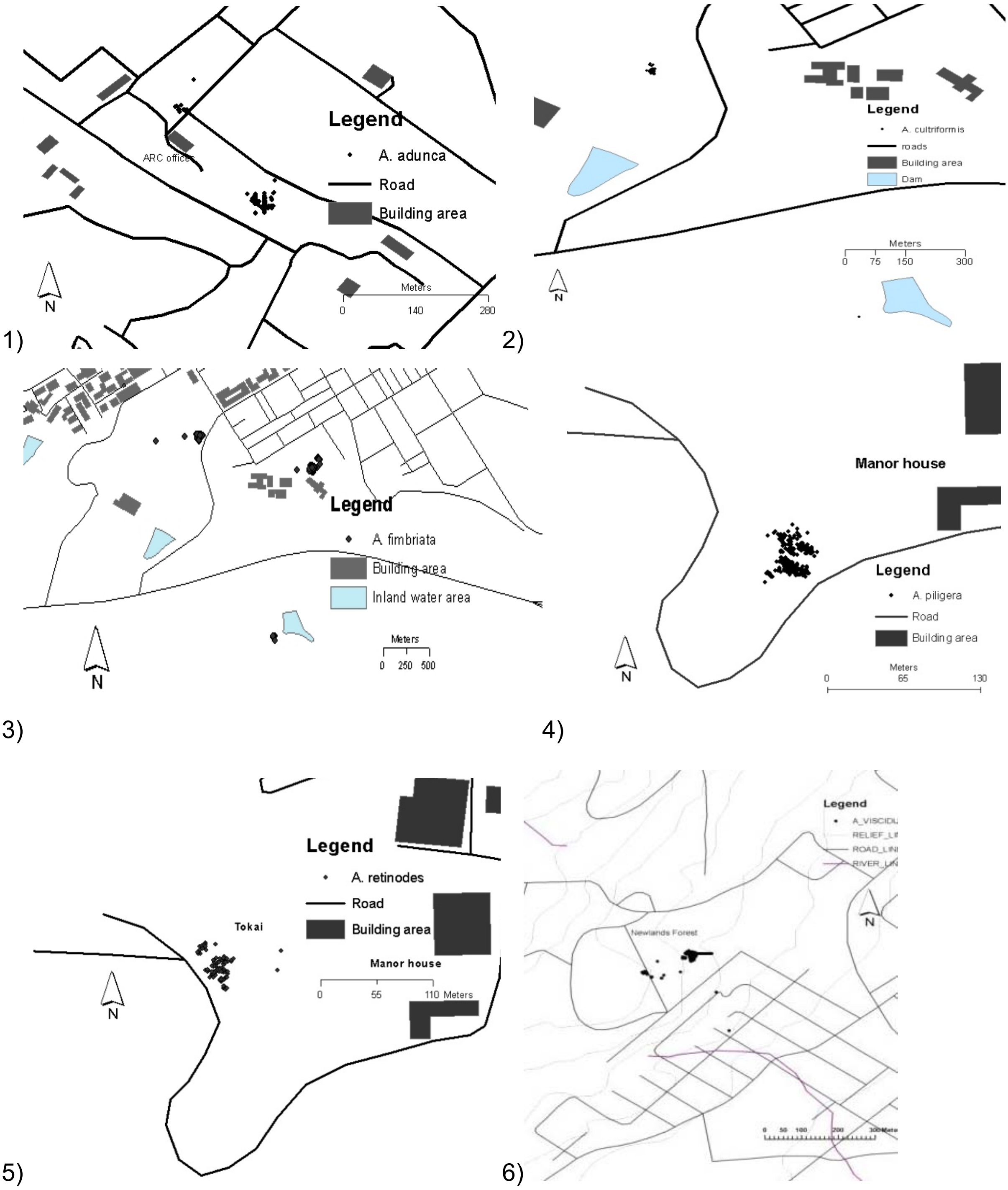
Wilson JR, Ivey P, Manyama P, Nänni I, (2013) A new national unit for invasive species detection, assessment and eradication planning. S Afr J Sci 109(5/6) http://dx.doi.org/10.1590/sajs.2013/20120111

Wilson JR, Panetta FD, Lindgren C (2016) Detecting and responding to alien plant incursions. Cambridge University Press, Cambridge

Worldwide Wattle ver. 2. Available online at: www.worldwidewattle.com Accessed15 August 2016. http://worldwidewattle.com/speciesgallery/piligera.php

Zenni RD, Wilson JRU, Le Roux JJ, Richardson DM (2009) Evaluating the invasiveness of *Acacia paradoxa* in South Africa. S Afr J Bot 75:485-496

Figure S 2.1. The distribution of selected naturalized Australian *Acacia* species in South Africa: 1) *A. adunca*; 2) *A. cultriformis*; 3) *A. fimbriata*; 4) *A. piligera*;5) *A retinodes*; 6) *A. viscidula*.



Supplementary Table 2.1: Molecular and morphological assessments of the identity of Australian *Acacia* species collected in South Africa. Blast uncertainty means the level of confidence with H indicating "high uncertainty": DNA sequencing data for voucher specimen that matches putative field identification not available and Blast hit with low statistical support; M medium uncertainty: DNA sequencing data for voucher specimen that matches putative field identification not available but Blast hit with high statistical support. Lastly, L means "low" uncertainty: DNA sequencing data for voucher specimen that matches putative field identification available and Blast hit with high sequence similarity and statistical support.

***Acacia* species (putative field identification)**	**Locality**	**Latitude**	**Longitude**	**ETS Genbank accession number**	**Putative species on Genbank?**	**BLAST uncertainty**	**Genbank ETS hit 1**	**Genbank ETS hit 2**	**Genbank ETS hit3**	**psbA-trnH Genbank accession number**	**Putative species on Genbank?**	**BLAST uncertainty**	**Genbank psbA-trnH hit 1**	**Genbank psbA-trnH hit 2**	**Genbank psbA-trnH hit 3**	**Notes**
A aneura	Paarl Arboretum	33.76129	18.9755	XXX	No	H	*Acacia aneura (99%)*	*Acacia ayersiana (99%)*	*Acacia hemiteles (99%)*	XXX	Y	H	*Acacia resinosa (100% & 100%)*	*Acacia coolgardiensis (100%)*	*Acacia crassicarpa (100%)*	Cannot conclusively confirm identity
A. adunca	Bien Donne Farm			XXX	No	M	*Acacia venulosa (99%)*	*Acacia aspera (98%)*	*Acacia elongata (98%)*	XXX	Y	H	*Acacia viscidula (100%)*	*Acacia flexifolia (94%)*	*Acacia cognata (94%)*	Based on psbA-trnH barcode is *A. viscidula*
A. adunca	33.8442	18.98197		XXX	No	H	*Acacia filicifolia (98%)*	*Acacia falciformis (98%)*	*Acacia neriifolia (98%)*	XXX	Y	H	*Acacia daphnifolia (95%)*	*Acacia neriifolia (99%)*	*Acacia colei (98%)*	Cannot conclusively confirm identity
A. cultriformis	Stellenbosch	33.05944	18.85141	XXX	Yes	L	*Acacia cultriformis (100%)*	*Acacia falciformis (99%)*	*Acacia dorothea (99%)*	XXX	Y	H	*Acacia colei (98%)*	*Acacia spectabilis (98%)*	*Acacia mearnsii (98%)*	Based putative field identification and ETS barcode is *A. cultriformis*
A. cultriformis	Colesberg	30.7248	25.09164	XXX	Yes	L	*Acacia cultriformis (100%)*	*Acacia falciformis (99%)*	*Acacia dorothea (99%)*	XXX	Y	H	*Acacia colei (98%)*	*Acacia spectabilis (98%)*	*Acacia mearnsii (98%)*	Based putative field identification and ETS barcode is *A. cultriformis*
A. cultriformis	Grahamstown Botanical Garden	33.95611	22.41167	XXX	Yes	L	*Acacia cultriformis (100%)*	*Acacia falciformis (99%)*	*Acacia penninervis (99%)*	XXX	Y	H	*Acacia colei (98%)*	*Acacia spectabilis (98%)*	*Acacia mearnsii (98%)*	Based putative field identification and ETS barcode is *A. cultriformis*
A. cultriformis	Bloemfontein			XXX	Yes	L	*Acacia cultriformis (100%)*	*Acacia falciformis (99%)*	*Acacia dorothea (99%)*	XXX	Y	H	*Acacia colei (99%)*	*Acacia mearnsii (98%)*	*Acacia dealbata (98%)*	Based putative field identification and ETS barcode is *A. cultriformis*

Acacia species (putative field identification)	Locality	Latitude	Longitude	ETS Genbank accession number	Putative species on Genbank?	BLAST uncertainty	Genbank ETS hit 1	Genbank ETS hit 2	Genbank ETS hit3	psbA-trnH Genbank accession number	Putative species on Genbank?	BLAST uncertainty	Genbank psbA-trnH hit 1	Genbank psbA-trnH hit 2	Genbank psbA-trnH hit 3	Notes
A. floribunda	University of the Witwatersrand	26.1571	27.99919	XXX	Yes	L	*Acacia mucronata (99%)*	*Acacia mucronata (99%)*	*Acacia mucronata (99%)*	XXX	Y	M	*Acacia longifolia (99%)*	*Acacia longifolia (99%)*	*Acacia restiacea (98%)*	Cannot conclusively confirm identity, definitely not *A. floribunda*
A. floribunda	Heidelberg	26.1571	27.99919	XXX	Yes	L	*Acacia mucronata subsp. mucronata (99%)*	*Acacia mucronata (99%)*	*Acacia mucronata (99%)*	XXX	Y	M	*Acacia phlebophylla (99%)*	*Acacia longifolia (98%)*	*Acacia longifolia (98%)*	Cannot conclusively confirm identity, definitely not *A. floribunda*
A. implexa	Grahamstown Botanical Garden	33.95611	22.4117	XXX	Yes	L	*Acacia implexa (99%)*	*Acacia implexa (99%)*	*Acacia melanoxylon (99%)*	XXX	Y	H	*Acacia maidenii (99%)*	*Acacia umbraculiformis (99% & 96%)*	*Acacia stereophylla var. stereophylla (99% & 98%)*	Based putative field identification and ETS barcode is *A. implexa*
A. pendula	Grootfontein, Middelburg	31.47095	25.027878	XXX	No	M	*Acacia mucronata (99%)*	*Acacia mucronata (99%)*	*Acacia mucronata (99%)*	XXX	na	na	na	na	na	Cannot conclusively confirm identity
A. retinodes	Tokai	33.05944	18.41507	XXX	Yes	L	*Acacia retinodes (99%)*	*Acacia saligna (99%)*	*Acacia retinodes (99%)*	XXX	Y	H	*Acacia beckleri (99%)*	*Acacia hakeoides (99%)*	*Acacia dealbata (98%)*	Based putative field identification and ETS barcode is *A. retinodes*
A. retinodes	Tokai	33.05944	18.41507	XXX	Yes	L	*Acacia retinodes (99%)*	*Acacia saligna (99%)*	*Acacia retinodes (99%)*	XXX	Y	H	*Acacia mearnsii (98%)*	*Acacia dealbata (98%)*	*Acacia dealbata (98%)*	Based ETS barcode and morphological resemblance of field identity is likely *A. doratoxylon*
A. salicina	Emmerantia	26.1571	27.99919	XXX	Yes	L	*Acacia salicina (99%)*	*Acacia bivenosa (99%)*	*Acacia tysonii (98%)*	XXX	Y	H	*Acacia xanthina (99%)*	*Acacia rostellifera (99%)*	*Acacia ashbyae (99%)*	Based ETS and psbA-trnH barcodes and morphology is *A. salicina*, a species known to have been introduced into South Africa (Damara farms)

Acacia species (putative field identification)	Locality	Latitude	Longitude	ETS Genbank accession number	Putative species on Genbank?	BLAST uncertainty	Genbank ETS hit 1	Genbank ETS hit 2	Genbank ETS hit3	psbA-trnH Genbank accession number	Putative species on Genbank?	BLAST uncertainty	Genbank psbA-trnH hit 1	Genbank psbA-trnH hit 2	Genbank psbA-trnH hit 3	Notes
A. ulicifolia	Tokai Arboretum	33.05944	18.41507	XXX	No	M	*Acacia aculeatissima (98%)*	*Acacia carnosula (93%)*	*Acacia longispinea (94%)*	XXX	N	M	*Acacia longispinea (98%)*	*Acacia longispinea (98%)*	*Acacia erinacea (97%)*	Cannot conclusively confirm identity
A. ulicifolia	Tokai	33.05944	18.41507	XXX	No	H	*Acacia aculeatissima (98%)*	*Acacia longispinea (93%)*	*Acacia carnosula (93%)*	XXX	N	M	*Acacia longispinea (97%)*	*Acacia longispinea (97%)*	*Acacia obtecta (97%)*	Cannot conclusively confirm identity
A. viscidula	Newlands Forest	33.9758	18.4431	XXX	No	M	*Acacia venulosa (99%)*	*Acacia aspera (98%)*	*Acacia elongata (98%)*	XXX	Y	L	*Acacia viscidula (100%)*	*Acacia flexifolia (94%)*	*Acacia cognata (94%)*	Based putative field identification and psbA-trnH barcode is *A. viscidula*
A. viscidula	Newlands Forest	33.9758	18.4431	XXX	No	M	*Acacia venulosa (99%)*	*Acacia aspera (98%)*	*Acacia elongata (98%)*	XXX	Y	L	*Acacia viscidula (99%)*	*Acacia cognata (94%)*	*Acacia baeuerlenii (94%)*	Based putative field identification and psbA-trnH barcode is *A. viscidula*
A. viscidula	Newlands Forest	33.9758	18.4431	XXX	No	M	*Acacia venulosa (99%)*	*Acacia aspera (98%)*	*Acacia elongata (98%)*	XXX	Y	L	*Acacia viscidula (100%)*	*Acacia flexifolia (94%)*	*Acacia cognata (94%)*	Certainly *A. viscidula*, also based on ETS similarity
Acacia adunca	33.8442	18.98197		XXX	No	H	*Acacia filicifolia (98%)*	*Acacia falciformis (98%)*	*Acacia neriifolia (98%)*	XXX	Y	H	*Acacia daphnifolia (95%)*	*Acacia neriifolia (99%)*	*Acacia colei (98%)*	Cannot conclusively confirm identity
Acacia fimbriata	Grahamstown	33.31813	26.52877	XXX	Yes	H	*Acacia neriifolia (100%)*	*Acacia falciformis (99%)*	*Acacia pustula (99%)*	XXX	Y	H	*Acacia daphnifolia (98%)*	*Acacia colei (98%)*	*Acacia stereophylla var. stereophylla (97%)*	Cannot conclusively confirm identity
Unknown *Acacia* species	Grootfontein, Middelburg	31.47095	25. 027878	XXX	na	M	*Acacia pendula (99%)*	*Acacia pendula (99%)*	*Acacia validinervia (99%)*	XXX	na	na	*Acacia cyclops (99%)*	*Acacia umbraculiformis (98%)*	*Acacia longiphyllodinea (98%)*	Cannot conclusively confirm identity
Unknown *Acacia* species	Newlands	33.97583	18.44306	XXX	na	M	*Acacia gonophylla (95%)*	*Acacia extensa (95%)*	*Acacia shuttleworthii (93%)*	XXX	na	na	na	na	na	Cannot conclusively confirm identity
Unknown *Acacia* species	Johannesburg Botanical Gardens	26.1571	27.99919	XXX	na	M	*Acacia hakeoides (99%)*	*Acacia hakeoides (99%)*	*Acacia hakeoides (99%)*	XXX	na	M	*Acacia hakeoides (99%)*	*Acacia hakeoides (99%)*	*Acacia beckleri (99%)*	Based ETS and psbA-trnH barcodes and morphology likely *A. hakeoides*

Acacia species (putative field identification)	Locality	Latitude	Longitude	ETS Genbank accession number	Putative species on Genbank?	BLAST uncertainty	Genbank ETS hit 1	Genbank ETS hit 2	Genbank ETS hit3	psbA-trnH Genbank accession number	Putative species on Genbank?	BLAST uncertainty	Genbank psbA-trnH hit 1	Genbank psbA-trnH hit 2	Genbank psbA-trnH hit 3	Notes
Unknown *Acacia* species	Springs	26.1571	27.99919	XXX	na	M	*Acacia koa (99%, 386/389)*	*Acacia koa (99%, 386/389)*	*Acacia koa (99%, 386/389)*	XXX	na	M	*Acacia confusa (99%)*	*Acacia melanoxylon (99%)*	*Acacia melanoxylon (99%)*	Based ETS and psbA-trnH barcodes and morphology is *A. melanoxylon*
Unknown *Acacia* species	Malmesbury	33.5136	18.63333	XXX	na	M	*Acacia ramulosa (100%) Acacia brachystachya (100%)*	*Acacia brachystachya (100%)*	*Acacia tumida (98%)*	XXX	na	L	*Acacia ramulosa var. ramulosa (100%)*	*Acacia sibina (98%)*	*Acacia diallaga (98%)*	Based ETS barcode and morphology is likely *A. ramulosa*
Unknown *Acacia* species	Malmesbury	33.5136	18.63333	XXX	na	H	*Acacia plectocarpa (98%)*	*Acacia curranii (96%)*	*Acacia delibrata (96%)*	XXX	na	na	na	na	na	Cannot conclusively confirm identity
Unknown *Acacia* species	Malmesbury	33.5136	18.63333	XXX	na	H	*Acacia stipuligera (97%)*	*Acacia torulosa (96%)*	*Acacia proiantha (97%)*	XXX	na		*Acacia yorkrakinensis subsp. acrita (99%)*	*Acacia ampliata (99%)*	*Acacia resinimarginea (99%)*	Cannot conclusively confirm identity
Unknown *Acacia* species	Malmesbury	33.5136	18.63333	XXX	Yes	L	*Acacia neriifolia (100%)*	*Acacia cupularis (99%)*	*Acacia pustula (99%)*	XXX	na		*Acacia neriifolia (99%)*	*Acacia dealbata (99%)*	*Acacia mearnsii (99%)*	Based ETS and psbA-trnH barcodes and morphology is *A. neriifolia*
Unknown *Acacia* species	Malmesbury	33.5136	18.63333	XXX	na	H	*Acacia salicina (99%)*	*Acacia bivenosa (99%)*	*Acacia tysonii (98%)*	XXX	na	na	na	na	na	Cannot conclusively confirm identity
Unknown *Acacia* species	Malmesbury	33.5136	18.63333	XXX	na	H	*Acacia aneura (99%)*	*Acacia hemiteles (99%)*	*Acacia ayersiana (98%)*	XXX	na	M	*Acacia resinosa (99%)*	*Acacia resinosa (99 & 100%)*	*Acacia coolgardiensis (99%)*	Cannot conclusively confirm identity
Unknown *Acacia* species	Malmesbury	33.5136	18.63333	XXX	na	H	*Acacia hemiteles (99%)*	*Acacia aneura (99%)*	*Acacia paraneura (99%)*	XXX	na	M	*Acacia resinosa (98%)*	*Acacia resinosa (98%)*	*Acacia effusifolia (98%)*	Cannot conclusively confirm identity
Unknown *Acacia* species	Malmesbury	33.5136	18.63333	XXX	na	H	*Acacia bivenosa (99%)*	*Acacia cupularis (99%)*	*Acacia tysonii (99%)*	XXX	na	M	*Acacia sclerosperma subsp. sclerosperma (100%)*	*Acacia sclerosperma subsp. sclerosperma (99%)*	*Acacia ligulata (99%)*	Cannot conclusively confirm identity
Unknown *Acacia* species	Malmesbury	33.5136	18.63333	XXX	na	na	na	na	na	XXX	na	M	*Acacia resinosa (100% & 100%)*	*Acacia coolgardiensis (100%)*	*Acacia crassicarpa (100%)*	Based on psbA-trnH and morphology likely *A. coolgardiensis*

Acacia species (putative field identification)	Locality	Latitude	Longitude	ETS Genbank accession number	Putative species on Genbank?	BLAST uncertainty	Genbank ETS hit 1	Genbank ETS hit 2	Genbank ETS hit3	psbA-trnH Genbank accession number	Putative species on Genbank?	BLAST uncertainty	Genbank psbA-trnH hit 1	Genbank psbA-trnH hit 2	Genbank psbA-trnH hit 3	Notes
Unknown *Acacia* species	Malmesbury	33.5136	18.63333	XXX	na	H	*Acacia tysonii (98%)*	*Acacia cupularis (98%)*	*Acacia bivenosa (98%)*	XXX	na	H	*Acacia stereophylla var. stereophylla (98%)*	*Acacia dorothea (98%)*	*Acacia jennerae (96%)*	Cannot conclusively confirm identity
Unknown *Acacia* species	Malmesbury	33.5136	18.63333	XXX	na	H	*Acacia aneura (99%)*	*Acacia ayersiana (99%)*	*Acacia hemiteles (99%)*	XXX	na	H	*Acacia coolgardiensis (99%)*	*Acacia abbreviata (99%)*	*Acacia diallaga (98%)*	Cannot conclusively confirm identity
Unknown *Acacia* species	Malmesbury	33.5136	18.63333	XXX	na	H	*Acacia elongata (97%)*	*Acacia baeuerlenii (96%)*	*Acacia aspera (97%)*	XXX	na	H	*Acacia inceana subsp. conformis (99% & 97%)*	*Acacia inceana subsp.conformis (99% & 97%)*	*Acacia cyclops (99% & 100%)*	Cannot conclusively confirm identity
Unknown *Acacia* species	Malmesbury	33.5136	18.63333	XXX	na	H	*Acacia sericophylla (99%)*	*Acacia coriacea (99%)*	*Acacia hamersleyensis (98%)*	XXX	na	H	*Acacia lasiocalyx (100% & 100%)*	*Acacia stereophylla var. stereophylla (99% & 100%)*	*Acacia lasiocalyx (99% & 100%)*	Cannot conclusively confirm identity
Unknown *Acacia* species	Malmesbury	33.5136	18.63333	XXX	na	H	*Acacia bivenosa (99%)*	*Acacia cupularis (99%)*	*Acacia tysonii (99%)*	XXX	na	H	*Acacia sclerosperma subsp. sclerosperma (100%)*	*Acacia xanthina (99%)*	*Acacia rostellifera (99%)*	Cannot conclusively confirm identity
Unknown *Acacia* species	Malmesbury	33.5136	18.63333	XXX	na	L	*Acacia neriifolia (100%)*	*Acacia falciformis (99%)*	*Acacia cupularis (99%)*	XXX	na	L	*Acacia neriifolia (99%)*	*Acacia dealbata (99%)*	*Acacia mearnsii (99%)*	Based ETS and psbA-trnH barcodes and morphology is *A. neriifolia*
Unknown *Acacia* species	Malmesbury	33.5136	18.63333	XXX	na	H	*Acacia confluens (99%)*	*Acacia tenuinervis (97%)*	*Acacia striatifolia (97%)*	XXX	na	H	*Acacia yorkrakinensis subsp. acrita (99%)*	*Acacia yorkrakinensis subsp. acrita (96%)*	*Acacia resinimarginea (96%)*	Cannot conclusively confirm identity
Unknown *Acacia* species	Malmesbury	33.5136	18.63333	XXX	na	H	*Acacia tysonii (98%)*	*Acacia cupularis (98%)*	*Acacia bivenosa (98%)*	XXX	na	na	na	na	na	Cannot conclusively confirm identity
Unknown *Acacia* species	Malmesbury	33.5136	18.63333	XXX	na	H	*Acacia elongata (97%)*	*Acacia baeuerlenii (96%)*	*Acacia aspera (96%)*	XXX	na	H	*Acacia inceana subsp. conformis (99%)*	*Acacia inceana subsp. conformis (99%)*	*Acacia sibina (98%)*	Cannot conclusively confirm identity
Unknown *Acacia* species	Malmesbury	33.5136	18.63333	XXX	na	L	*Acacia acuminata (100%)*	*Acacia acuminata (100%)*	*Acacia acuminata (100%)*	XXX	na	H	*Acacia acuminata (99%)*	*Acacia stereophylla var. stereophylla (99%)*	*Acacia burkittii (99%)*	Cannot conclusively confirm identity
Unknown *Acacia* species	Malmesbury	33.5136	18.63333	XXX	na	H	*Acacia drepanophylla (99%)*	*Acacia denticulosa (97%)*	*Acacia sessilispica (97%)*	XXX	na	H	*Acacia stereophylla var. stereophylla (98%)*	*Pithecellobium clypearia (98%)*	*Acacia acuminata (98%)*	Cannot conclusively confirm identity

Acacia species (putative field identification)	Locality	Latitude	Longitude	ETS Genbank accession number	Putative species on Genbank?	BLAST uncertainty	Genbank ETS hit 1	Genbank ETS hit 2	Genbank ETS hit3	psbA-trnH Genbank accession number	Putative species on Genbank?	BLAST uncertainty	Genbank psbA-trnH hit 1	Genbank psbA-trnH hit 2	Genbank psbA-trnH hit 3	Notes
Unknown *Acacia* species	Malmesbury	33.5 136	18.63333	XXX	na	M	*Acacia murrayana (100%)*	*Acacia murrayana (100%)*	*Acacia murrayana (99%)*	XXX	na	L	*Acacia murrayana (99%)*	*Acacia murrayana (99%)*	*Acacia umbraculiformis (97%)*	Based ETS and psbA-trnH barcodes and morphology is *A. murrayana*
Unknown *Acacia* species	Malmesbury	33.5 136	18.63333	XXX	na	L	*Acacia hakeoides (99%)*	*Acacia hakeoides (99%)*	*Acacia hakeoides (99%)*	XXX	na	L	*Acacia hakeoides (100%)*	*Acacia hakeoides (99%)*	*Acacia beckleri (99%)*	Based ETS and psbA-trnH barcodes and morphology likely *A. hakeoides*
Unknown *Acacia* species	Malmesbury	33.5 136	18.63333	XXX	na	H	*Acacia concurrens (99%)*	*Acacia pellita (99%)*	*Acacia concurrens (99%)*	XXX	na	na	na	na	na	Cannot conclusively confirm identity
Unknown *Acacia* species	Malmesbury	33.5 136	18.63333	XXX	na	H	*Acacia bivenosa (99%)*	*Acacia tysonii (99%)*	*Acacia rostellifera (99%)*	XXX	na	H	*Acacia sclerosperma subsp. sclerosperma (99%)*	*Acacia sclerosperma subsp. sclerosperma (99%)*	*Acacia xanthina (99%)*	Cannot conclusively confirm identity
Unknown *Acacia* species	Malmesbury	33.5 136	18.63333	XXX	na	H	*Acacia drepanophylla (99%)*	*Acacia denticulosa (96%)*	*Acacia neurophylla (96%)*	XXX	na	H	*Acacia stereophylla var. stereophylla (98%)*	*Acacia dorothea (98%)*	*Acacia jennerae (96%)*	Cannot conclusively confirm identity
Unknown *Acacia* species	Malmesbury	33.5 136	18.63333	XXX	na	M	*Acacia neriifolia (99%)*	*Acacia falciformis (99%)*	*Acacia cupularis (99%)*	XXX	na	M	*Acacia neriifolia (99%)*	*Acacia pustula (100%)*	*Acacia dealbata (99%)*	Based ETS and psbA-trnH barcodes and morphology is *A. neriifolia*
Unknown *Acacia* species	Malmesbury	33.5 136	18.63333	XXX	na	M	*Acacia hakeoides (100%)*	*Acacia hakeoides (100%)*	*Acacia hakeoides (100%)*	XXX	na	H	*Acacia hakeoides (99%)*	*Acacia hakeoides (99%)*	*Acacia beckleri (99%)*	Based ETS and psbA-trnH barcodes and morphology likely *A. hakeoides*
Unknown *Acacia* species	Malmesbury	33.5 136	18.63333	XXX	na	H	*Acacia calcicola (99%)*	*Acacia calcicola (100%)*	*Acacia calcicola (99%)*	XXX	na	H	*Acacia yorkrakinensis subsp. Acrita (94%)*	*Acacia inceana subsp.conformis (99 & 98%)*	*Acacia umbraculiformis (99 & 98%)*	Cannot conclusively confirm identity

S2.2. Information about the Acacia species planted in Damara Farm.

The forestry trial on Damara Farm in the Western Cape significantly increased the number of species known to have been introduced and that are still present in South Africa. The possible presence of other such trials on private land represent a major source of uncertainty when compiling alien plant lists.

The trial plantation at Damara farm was part of Forestry Faculty of the University of Stellenbosch to plant trees at six dry land trial locations. The aim of the trial, plantations on the West Coast of South Africa, was to investigate tree species that would be planted for commercial purposes. In 1997, it was decided to extend the existing dryland trials into research in agroforestry system that could be used by small farmers (Fig. 1). Then, Mr Armstrong (owner of the Damara farm) was approached to give advice about how to conduct these trials, since he had experience in tree planting and it was also felt that he would also provide land to do these trials. In 1998, seed of 33 Australian acacias provided by the Department of Environmental Affairs was planted at Damara farm. Based on the observed populations at Damara farm, 3 species had pods and seed with seedlings underneath and some were suckering, I proposed as B2 'individuals transported beyond limits of native range, and in cultivation (i.e. individuals provided with conditions suitable for them, but explicit measures to prevent dispersal are limited at best)'. 6 species with no pods or flowers I proposed as B2 'individuals transported beyond limits of native range, and in cultivation (i.e. individuals provided with conditions suitable for them, but explicit measures to prevent dispersal are limited at best). 17 species had flowers or pods and a biological agent, I proposed as B2 'individuals transported beyond limits of native range, and in cultivation (i.e. individuals provided with conditions suitable for them, but explicit measures to prevent dispersal are limited at best)'

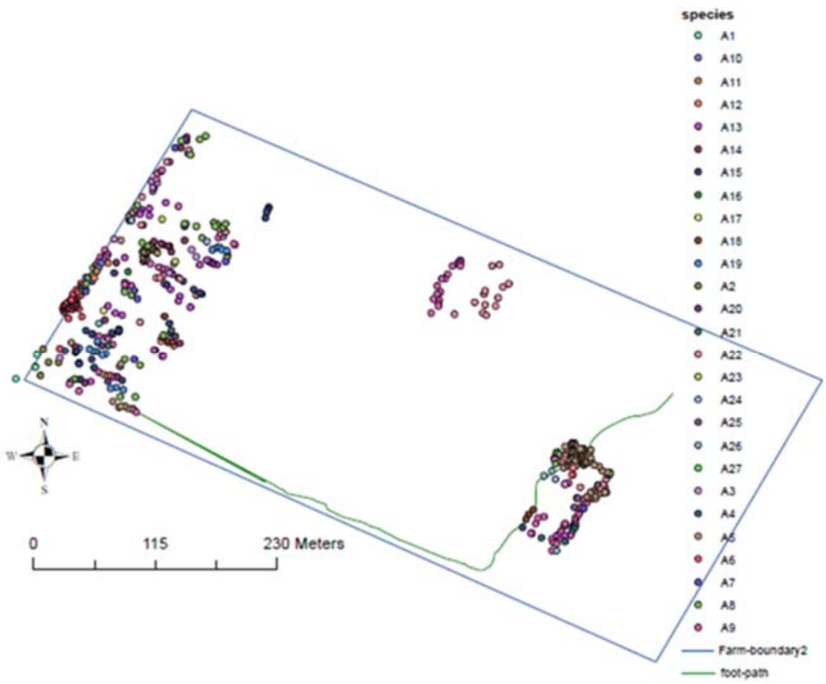


Fig S2.2

Fig S2.2

S3.1. Species information for the six naturalized wattles in South Africa.

Acacia adunca occurs as a native species along the Great Dividing Range from Bolivia Hill to Legume, NSW, and along the south-eastern Queensland border in Australia (Worldwide Wattle 2016). The species occurs in forests and woodlands on sandy-loam and granitic derived soils. Trees reach about 5-6m high and have narrowly linear phyllodes that are often wrinkled when dry. Bright yellow flowers occur in axillary racemes during July to October. The seed pods are often slightly curved and the seed funicle is expanded. In South Africa this species is only known to be naturalized at the Bien Donne experimental farm outside Paarl in the Western Cape (Fig. 3.2) (Wilson et al. 2010).

Acacia cultriformis is native to Australia where it is cultivated as an ornamental plant in parks and gardens (Worldwide Wattle, 2016). The species has bright yellow flowers that appear from August to November in its natural range. Branchlets may be bare and smooth or covered with a white bloom. Phyllodes, which are crowded along the stems, are green to green-grey and are irregular, with one leaf margin angled so the overall shape is triangular. *Acacia cultriformis* has become invasive in Southern California (http://www.ucjeps.berkeley.edu/consortium/) and is naturalized in Ethiopia, Zimbabwe, South Africa, India, Indonesia, Brazil and Argentina http://www.inaturalist.org/taxa/181887-Acacia-cultriformis. (Accessed on 15 March 2016). In South Africa, the species was first cultivated in Cape of Good Hope in 1858 and later in 1885 the plants were cultivated in the nursery at the Tokai Arboretum, Lichtenburg plantation and at Potchefstroom Agricultural College. In 2014, one plant was found near Gray dam in Grahamstown (Pers. obs); herbarium records indicate that the species was cultivated in the Grahamstown Botanical Garden. In 2015, many individuals were discovered in the Makana Botanical Garden (Grahamstown) (pers. obs).

Acacia fimbriata is widespread in eastern Australia, mainly in coastal regions of Queensland and New South Wales (Worldwide Wattle, 2016). The species occurs predominantly along streams and margins of rainforest from Nerringa in New South Wales to Carnarvon National Park and Ravenshoe in Queensland. The species grows to about 6m high and has linear to narrowly elliptic, slightly curved phyllodes. It has bright golden and sometimes yellow flowers that occur from July to November. The pods are firmly chartaceous and glabrous and seeds are black and shiny (Flora of Australia, 2001; Poynton 2009). This species is only known to be naturalized at three sites in Grahamstown (Philip Weyl, pers. obs.).

According to Motloung et al. (2014), a large proportion of southern Africa is a climatically suitable range for all of the above-mentioned species.

Acacia piligera (putative name, see Chapter 2) is native to the upper Hunter Valley southwards towards the Hunter Range, New South Wales, Australia (Worldwide Wattle, 2016). Plants are obconical, open shrubs up to 1.5-2m tall with branches more or less erect or curving upwards. Phyllodes are grey-green to green and more or less straight. Pods are mostly curved, 3-8cm long, 16-30mm wide, leathery to firm with margins that are usually undulate. Flowers are mid-yellow; fruits are pale dull green with maroon shade. In 2014, the species was found to have naturalized in the Tokai forest on the Cape Peninsula, Western Cape, South Africa. It is not yet listed under the NEMBA Regulations as an invasive alien species**.** Given the widespread invasions of other similar Australian *Acacia* species in South Africa, *A. piligera* has the potential to become a significant threat to biodiversity.

Acacia retinodes occurs discontinuously from the Eyre Peninsula in South Australia to Wilson's Promontory in Victoria and as far south as Tasmania. It grows mainly in poorly drained soils inland from the coast. The species reaches a height of 10 m and has oblanceolate phyllodes and pale yellow flowers. The pods are linear and firmly to thinly chartaceous. Funicles encircle seeds in the pod (Worldwide Wattle, 2009). The species is invasive in Portugal and Hawaii (Richardson and Rejmánek, 2011). The species was first recorded on the Cape Flats in 1865 (Poynton, 2009), and has an established population in the Tokai section of Table Mountain National Park (Poynton 2009; Wilson et al. 2010).

Acacia viscidula occurs in the Darling Downs in south-eastern Queensland and adjoining New South Wales (Worldwide wattle, 2016). The species grows predominantly in dry sclerophyll forests with granitic soils. It grows to about 3-4m high and has straight to slightly curved leaves. Flowering normally occurs in the late spring with light golden flowers. The pods are linear, raised over the seeds and dark brown (Flora of Australia, 2001). It has linear incurved ascending sticky phyllodes (hence, the common name sticky wattle) because of glabrous with three to seven distant impressed resinous nerves. In South Africa, this species is naturalized in the Newlands Forest section of the Table Mountain National Park (Poynton, 2009; Wilson et al. 2010) and on adjacent neighbourhood on the streets.

S3.2. R- code and generalized linear model (with poisson errors), indicating influence of each treatment on germination of *Acacia* seeds.

```
##Adunca generalized linear model
> data<-read.csv(file.choose(),header=T)
> data$Treatment<-relevel(data$Treatment,ref="Control")
> levels(data$Treatment)
[1] "Control"                "100 degrees"            "100 degrees with sm
oke"
[4] "60 degrees"             "Smoke"
> glm1 = glm(Gm~Treatment,data=data,family=poisson)
> summary(glm1)

Call:
glm(formula = Gm ~ Treatment, family = poisson, data = data)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-4.6368  -0.6013   0.2238   1.0143   1.7172

Coefficients:
                                Estimate Std. Error z value Pr(>|z|)
(Intercept)                       2.0477     0.1796  11.401  < 2e-16 ***
Treatment100 degrees              0.5363     0.2261   2.372 0.017698 *
Treatment100 degrees with smoke   0.3272     0.2356   1.389 0.164903
Treatment60 degrees               0.1769     0.2435   0.727 0.467435
TreatmentSmoke                   -2.0477     0.5313  -3.854 0.000116 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for poisson family taken to be 1)
```

```
    Null deviance: 111.933  on 19  degrees of freedom
Residual deviance:  57.294  on 15  degrees of freedom
AIC: 133.51

Number of Fisher Scoring iterations: 5

##Acacia fimbriata GERALIZED LINEAR MODEL
> fimbr<-read.csv(file.choose(),header=T)
data$Treatments<-relevel(data$Treatments,ref="Control")
> levels(data$Treatments)
[1] "Control"                "100 degrees"
[3] "100 degrees with smoke" "60 degrees"
[5] "60 degrees with smoke"  "Smoke"
> fimbrglm = glm(Gm~Treatments,data=data,family=poisson)
> summary(fimbrglm)

Call:
glm(formula = Gm ~ Treatments, family = poisson, data = data)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-1.8990  -0.7071  -0.2692   0.5413   1.2221

Coefficients:
                                   Estimate Std. Error z value Pr(>|z|)
(Intercept)                      -1.386e+00  9.999e-01  -1.386  0.16563
Treatments100 degrees             4.205e+00  1.007e+00   4.174 2.99e-05***
Treatments100 degrees with smoke  4.304e+00  1.007e+00   4.276 1.91e-05***
Treatments60 degrees              2.833e+00  1.029e+00   2.754  0.00589**
Treatments60 degrees with smoke   2.944e+00  1.026e+00   2.870  0.00410**
TreatmentsSmoke                  -3.925e-15  1.414e+00   0.000  1.00000


Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for poisson family taken to be 1)

    Null deviance: 208.353  on 23  degrees of freedom
Residual deviance:  15.339  on 18  degrees of freedom
AIC: 95.107

Number of Fisher Scoring iterations: 5

##Acacia piligera GENERALIZED LINEAR MODEL

> pili<-read.csv(file.choose(),header=T)
> pili<-read.csv(file.choose(),header=T)
> data$Treatments<-relevel(data$Treatments,ref="Control")
> levels(data$Treatments)
[1] "Control"                "100 degrees"            "100 degrees with sm
oke"
[4] "60 degrees"             "60 degrees with smoke"  "Smoke"
> piliglm = glm(Gm~Treatments,data=data,family=poisson)
> summary(piliglm)

Call:
glm(formula = Gm ~ Treatments, family = poisson, data = data)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-1.8990  -0.7071  -0.2692   0.5413   1.2221
```

```
Coefficients:
                                   Estimate Std. Error z value Pr(>|z|)
(Intercept)                      -1.386e+00  9.999e-01  -1.386  0.16563
Treatments100 degrees             4.205e+00  1.007e+00   4.174 2.99e-05 **
*
Treatments100 degrees with smoke  4.304e+00  1.007e+00   4.276 1.91e-05 **
*
Treatments60 degrees              2.833e+00  1.029e+00   2.754  0.00589 **
Treatments60 degrees with smoke   2.944e+00  1.026e+00   2.870  0.00410 **
TreatmentsSmoke                  -3.925e-15  1.414e+00   0.000  1.00000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for poisson family taken to be 1)

    Null deviance: 208.353  on 23  degrees of freedom
Residual deviance:  15.339  on 18  degrees of freedom
AIC: 95.107

Number of Fisher Scoring iterations: 5
```