

**Aspects influencing the suitability of
Rhizaspidiotus donacis (Leonardi) (Hemiptera:
Diaspididae), a candidate biological control agent
for the invasive giant reed, *Arundo donax* L.
(Poaceae) in South Africa**

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PREFACE

The research contained in this dissertation was conducted by the candidate from April 2015 to November 2016, at the facilities of the Agricultural Research Council – Plant Protection Research Institute (ARC-PPRI) at Cedara and the School of Life Sciences, University of KwaZulu-Natal, Pietermaritzburg. The study was conducted under the supervision of Dr T. Olckers and the co-supervision of Dr A. Bownes.

This research represents original work by the author and has not been submitted in any form for any other degree or diploma to any University. The use of any other author's works has been duly acknowledged within the text.

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ABSTRACT

Arundo donax L. (Poaceae), is a perennial grass reed which is considered to be native to North Africa, India, the Middle East, the Mediterranean and the Persian Gulf, and is highly invasive and widespread in the United States of America (USA), Australia, Mexico and South Africa. The Agricultural Research Service of the United States Department of Agriculture (USDA-ARS) has initiated a research programme on biological control of *A. donax*, and has since released two agents; the armoured scale insect, *Rhizaspidiotus donacis* (Leonardi) (Hemiptera: Diaspididae) and the Arundo wasp, *Tetramesa romana* Walker (Hymenoptera: Eurytomidae). Following progress in the USA, the rhizome-, leaf-, and stem-feeding armoured scale insect, *R. donacis* is being considered for biological control of *A. donax* in South Africa. This study seeks to evaluate several aspects which may influence the suitability of *R. donacis* from Alicante, Spain for biological control of *A. donax* in South Africa.

An important component of pre-release evaluations in weed biological control programmes is to evaluate the climatic niches of candidate agents and the target weed to identify areas that are climatically suitable for their persistence in areas of introduction. The first aspect of the project evaluated whether *A. donax* has spread to all suitable areas in South Africa and whether South Africa is climatically suitable to support *R. donacis* populations. The climate-modelling programme CLIMEX was used to model climatic similarities between native range locations of *R. donacis* and South Africa, and the climatic suitability of South Africa for *R. donacis* and *A. donax*, using model parameters based on both native and introduced range (i.e. USA) distribution data. The results suggested that *A. donax* has reached its fundamental range in the Mediterranean, humid subtropical, marine west coastal and semi-arid climatic regions of South Africa. Furthermore, the results indicated that most regions of South Africa in which *A. donax* is present and abundant are climatically suitable for *R. donacis* establishment or are climatically similar to locations in the native range where the scale insect is established. Consequently, the climatic conditions in South Africa are likely to support biological control of *A. donax* using *R. donacis*.

The second aspect of this study was conducted in a quarantine environment and evaluated the suitability of *R. donacis* from Alicante, Spain for biological control of *A. donax* in South Africa, by measuring survival levels of *R. donacis* on *A. donax* and the effect of herbivory by *R. donacis* on *A. donax*'s growth parameters. The results showed low levels of establishment of *R. donacis* from Alicante, Spain on *A. donax* from South Africa and no

significant impact on the growth parameters of *A. donax*. These findings suggest probable poor host-plant matching and if this is the case, *R. donacis* from Alicante, Spain is likely to be ineffective in controlling South African *A. donax* populations. It is recommended that plant molecular studies be conducted to determine which regions in Spain, or elsewhere in the Mediterranean, harbour *A. donax* that is a genetically close match to South African *A. donax*. *Rhizaspidiotus donacis* stocks which are genetically better matched with *A. donax* in South Africa, are likely to perform better than those sourced from Alicante, Spain which proved ineffective. However, since the biocontrol programme is relatively novel with no previous experience in working with *R. donacis*, survival levels could have been compromised by the required conditions for development and breeding not being fully met in quarantine. It is recommended that a study be conducted to determine settlement levels, under differing planting conditions and watering regimes, when the next *R. donacis* consignment is received.

The climate of South Africa is favourable for biological control of *A. donax* in South Africa by the candidate agent, *R. donacis*. However, success is only likely to be realised if *R. donacis* is sourced from a region in Spain, or elsewhere in the Mediterranean, with *A. donax* populations that are a genetically close match for South African *A. donax*. Further investigations will be conducted on *R. donacis* from Alicante, Spain before it is ruled out as a promising biotype. However, plant molecular studies need to be conducted as a priority to identify *A. donax* populations that are a genetically close match to South African *A. donax*. This, as well as settlement and establishment trials with *R. donacis* from Alicante, Spain should constitute the focus of future research for the programme.

Key words: Agent impact; climate modelling; host acceptability; scale insects, weed biocontrol

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CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

1.1. INVASIVE ALIEN PLANTS

The high number of invasive alien plant (IAP) species in South Africa creates a prominent and difficult challenge (Moran *et al.* 2013). According to Richardson *et al.* (2000), invasion occurs when an introduced plant produces reproductive offspring in regions which are distant from sites of introduction and have the potential to spread over a considerable area. Plant invasions can take place in mature, undisturbed communities which have undergone complete succession, or in disturbed communities (Richardson *et al.* 2000). Alien plant invasions sometimes occur as a result of unintentional introduction, but most often take place due to intentional introductions by humans (Culliney 2005). Reasons for intentional introductions include use for screening, sand binding and stabilisation, security hedging, fodder, food, firewood, shelter, timber, erosion control and ornamental purposes (Henderson 2001; Mgidi *et al.* 2007).

Invasive alien plants are characterised as plant species which can easily overcome environmental or geographical barriers and rapidly expand and establish in areas outside of their native range. There are several common features which are characteristic of invasive weeds (Bromilow 1995; Culliney 2005). They reach maturity and reproductive stages quickly and seedling growth times are short. Reproduction which takes place vegetatively or through seed production is rapid and efficient. Likewise, dispersal can take place rapidly (McKinney & Lockwood 1999) and efficiently and special adaptations for both short and long distance dispersal often exist (Baker 1965; Bromilow 1995; Culliney 2005; Van Kleunen *et al.* 2015). Furthermore, IAPs are able to tolerate and persist in a variety of environmental and climatic conditions. They are able to produce high numbers of seeds in favourable environmental conditions and some seed in a broad range of environmental conditions (Culliney 2005). Similarly, no special environmental conditions are required for germination to occur (Baker 1965; Culliney 2005; Van Kleunen *et al.* 2015). In many cases, their persistence under adverse environmental conditions is promoted by rhizomes and vegetative organs which provide substantial food reserves and allow for vigorous growth (Culliney 2005). IAPs often have well-developed and deep rhizome systems (Bromilow 1995). Furthermore, they are usually able to regenerate rapidly when their vegetative organs have been cut (Culliney 2005).

Although there are ‘benign’ alien plants which may have no significant adverse impacts, IAPs are considerably more problematic (Richardson *et al.* 2000). According to Richardson *et al.* (2000), 50 to 80% of IAP species can be considered as problematic due to their harmful impacts. Invasive alien plants are considered to contribute significantly to global environmental change and have negative economic and environmental impacts worldwide (Vilà *et al.* 2011; Seastedt 2015). Approximately 10% of IAPs have the potential to substantially transform the condition and character of invaded ecosystems and require management and control (Richardson *et al.* 2000). Approximately 200 species are listed as detrimental to natural, protected ecosystems as well as pastoral and agricultural environments in South Africa (Richardson & Van Wilgen 2004; Moran *et al.* 2013), and IAPs in the country’s freshwater and terrestrial ecosystems constitute a significant environmental problem (Chamier *et al.* 2012).

1.1.1. Impacts of invasive alien plants

Invasive alien plants have significant environmental impacts. The most common impact is the reduction of native species due to increased competition for resources (Culliney 2005). Plant invasions are a major threat to both animal and plant biodiversity (Clewley *et al.* 2012), are the primary cause of avifaunal extinctions, and play a significant role in mammal and freshwater fish extinctions (Kannan *et al.* 2013). A rigorous review of the impacts of IAPs revealed that the majority of alien plant invasions led to decreased diversity of local plant species but an increase in overall plant production in the community (Vilà *et al.* 2011). In South Africa, 55% of Red Data listed plants and 60% of endemic freshwater fish species are threatened by IAPs (Kannan *et al.* 2013).

Both the quantity and quality of water resources can be impacted by IAPs (Enright 2000; Chamier *et al.* 2012). The impact of some IAPs on water quality begins with the change in plant biomass levels and distribution, which results in altered fire regimes, often increasing their intensity, which in turn increases soil erosion and reduces water quality. Many aquatic IAPs impact water quality by forming dense mats which impede the flow of water, causing lower oxygen concentrations and increasing organic matter levels. These conditions can lead to increased accumulation of sediment, and accelerate the rate of eutrophication which makes the water toxic to many terrestrial and aquatic species (Chamier *et al.* 2012). Water quantity impacts are often manifested by reduced river flows and increased evaporation rates as a consequence of the presence of IAPs. Furthermore, IAPs can impact ground water quality by

increasing nutrient concentrations (Chamier *et al.* 2012). In South Africa, IAPs in catchment areas affect entire water resource systems (Enright 2000), and water consumption by a number of invasive plant species far exceeds that of indigenous plant species (Moran *et al.* 2013). It is estimated that the total flow reduction is approximately 1444 million m³.yr⁻¹ or almost 2.9% of the naturalised mean annual runoff (Le Maitre *et al.* 2016).

IAPs have the potential to alter the natural cycles and ecological processes of native habitats. They have been identified by the Millennium Ecosystem Assessment (2005) as one of the major drivers of ecosystem change, having the potential to alter the structure, composition and function of ecosystems (Kannan *et al.* 2013). They alter ecosystem structures as they change the dynamics of food webs (Culliney 2005; Clewley *et al.* 2012). The complexity of their impacts is great as their magnitude and direction can vary at different ecological levels, including species, community and ecosystem levels (Vilà *et al.* 2011).

A global evaluation of the economic costs of the adverse impacts of IAP species was found to be approximately 1.4 trillion US dollars per year (Kannan *et al.* 2013). The economic costs resulting from IAPs in South Africa is equivalent to R6.5 billion per annum, approximately 0.3% of the country's gross domestic product (Chamier *et al.* 2012). IAP's adversely impact the economics of sectors such as forestry, fisheries, agriculture and livestock grazing as a result of their impact on ecological functioning (Clewley *et al.* 2012; Kannan *et al.* 2013). Invasive plants incur many costs to the agricultural sector and decrease profit margins as the cost of mechanical or chemical control increases crop production costs. Agricultural crop yields are often reduced due to competition with invasive plants for sunlight and nutrients or due to allelopathic effects (Culliney 2005).

1.1.2. Management of invasive alien plants

The management of alien plant invasions entails three main approaches; prevention, eradication and control. Control entails managing the extent of the invasion to reduce the environmental and economic impacts. There are three main methods of control, namely chemical control, mechanical control and biological control (Culliney 2005). Mechanical control involves the physical removal of invasive plants by harvesting, hand-pulling, tilling, hoeing, mowing, bulldozing, draining or a number of other means. Chemical control entails the use of artificially-produced herbicides such as respiration inhibitors, photosynthesis inhibitors, amino acid biosynthesis inhibitors, lipid biosynthesis inhibitors, cell division inhibitors and auxin mimics (Culliney 2005; Chalak *et al.* 2011). Biological control entails the

use of tested and approved natural enemies, notably insects or pathogens, to control invasive weed populations in the area of introduction, by releasing them in the invaded range and allowing them to establish (Culliney 2005; Clewley *et al.* 2012). Mechanical and chemical control can be effective in controlling small populations of invasive weeds, but repeated applications and larger weed populations render them labour intensive and uneconomical (Culliney 2005). Biological control provides an additional management option, where mechanical and chemical control methods are ineffective or insufficient (Culliney 2005; Clewley *et al.* 2012). The introduction of natural enemies (biological control agents) can help to maintain or reduce invasive weed populations to levels that are economically insignificant (Culliney 2005).

Innovation and investment in weed biological control research in South Africa has been motivated by the extensive adverse effects of IAPs in the country (Moran *et al.* 2013). The Agricultural Research Council - Plant Protection Research Institute (ARC-PPRI) drives biological control research efforts in the country, along with university partners (i.e. University of Cape Town, Rhodes University, University of Witwatersrand and University of KwaZulu-Natal), whilst the Working for Water programme (Department of Environmental Affairs) is largely responsible for carrying out chemical and mechanical control operations in the field. The operations of Working for Water are at an implementation level and work with government departments, conservation agencies, municipalities, forestry companies and other organisations to carry out their responsibilities of clearing invasive species. Most of the programme's funds are expended on the control of terrestrial IAPs. The programme has been able to clear several large areas of IAPs, mostly due to its success in securing substantial funding (van Wilgen & Wannenburgh 2016). Furthermore, it has provided political and financial support for biological control efforts and has incorporated biological control practices with chemical and mechanical control efforts, with much success. Additionally, the programme has helped to raise awareness of invasive alien species and has made investments into several relevant research areas (van Wilgen & Wannenburgh 2016).

Large investments in mechanical and chemical control by the Working for Water programme have only managed to slow down, rather than reverse invasions (Moran *et al.* 2013). This highlights the great need for greater investment into weed biological control research and implementation (Moran *et al.* 2013). Due to the levels of invasion or environmental problems associated with other control methods, management institutions often have no other option but to use biological control (Seastedt 2015). Biological control can

provide a feasible and effective solution, when mechanical and chemical control methods are inadequate (Van Wilgen *et al.* 2013). The approach is long term and self-sustaining in nature, favouring its use over mechanical and chemical control methods. An additional motivation for its use is its ability to provide effective control, particularly in environments which are more sensitive, such as riparian habitats (McFadyen 1998; Clewley *et al.* 2012).

1.2. BIOLOGICAL CONTROL

Weed biological control entails the use of biological control agents, either herbivorous arthropods or pathogens, which are natural enemies of the invasive plant species, and which have been evaluated for suitability, to feed on or damage the target species, reduce their densities and thereby reduce their adverse impacts (Moran *et al.* 2013; Seastedt 2015). It is based on the principle that introduced plants become invasive because of the absence of their natural enemies in the areas where they have invaded. Consequently, the introduction of biological control agents into the invaded range can help to regulate invasive plant populations. These agents may control weeds indirectly by imposing stresses on the target plant, thereby reducing their competitive advantage, or directly by damaging essential plant parts leading to the death of the plant or a reduction in its reproduction (Culliney 2005). This control method aims to decrease the fitness of a target plant to a level at which it is not an economic or ecological concern (McFadyen 1998; Culliney 2005; Seastedt 2015). It can be considered as an attempt to manipulate and restructure biotic communities in order to achieve conservation goals and help enhance ecosystem services (McFadyen 1998; Seastedt 2015).

The field of biological control is accountable to funders and future generations to make ecologically and economically sound choices, and biological control views and decisions always come down to issues of safety and effectiveness (Briese *et al.* 2003). There is a growing need for biological control practitioners to identify and prioritise agents which are most likely to be effective, prior to release (Sheppard 2003; Morin *et al.* 2009). An agent is effective if it is able to establish in the area of introduction and maintain population densities which are able to cause substantial damage to critical life stages of a target weed (McFadyen 2003). Pre-release efficacy assessments of candidate agents aid the selection of agents (Goolsby *et al.* 2004b; Morin *et al.* 2009). These assessments of agent efficacy are typically conducted in a glasshouse or laboratory or in the field (Morin *et al.* 2009). Often practitioners select agents

which can be reared easily, cause significant damage in the field, are host specific (McFadyen 2003), and are able to survive in the climate of the introduced region.

Arundo donax (L.) (Poaceae) is one example of an invasive weed which is currently being subjected to biological control in the United States of America (USA) (Goolsby *et al.* 2016). Furthermore, in South Africa, host-range and pre-release efficacy evaluations of prospective agents are currently being conducted to deal with *A. donax* invasions, using expertise developed in the USA.

1.3. ARUNDO DONAX

Arundo donax is a perennial grass reed which has been listed as one of the top 100 invasive species in the world by the World Conservation Union (Lowe *et al.* 2000). For hundreds of years, this rhizomatous grass has been used and introduced by humans to many parts of the world for the production of roof thatching, baskets, mats, walking sticks, woodwind instruments, training stakes and fishing canes (Mariani *et al.* 2010; Moran & Goolsby 2010). In addition, the high biomass productivity and stable long-term yields of *A. donax* cultivations favour its use as a bioenergy crop (Angelini *et al.* 2009), with major cultivations in Italy, southern Europe, Florida (USA) and Mediterranean areas (Angelini *et al.* 2009; Mariani *et al.* 2010; Moran 2015).

The success of *A. donax* as an invader can be partly attributed to its high environmental tolerance (Quinn & Holt 2008). The reed occupies wetlands and grasslands found in a wide range of climatic zones (Mariani *et al.* 2010). Plants are able to tolerate high levels of Cadmium (Cd) and Nickel (Ni), suggesting that they are able to grow in contaminated soils (Papazoglou *et al.* 2005; Papazoglou 2007). *Arundo donax* has a low tolerance for drought conditions and a higher tolerance for flood conditions (Mann *et al.* 2013). The plant's sensitivity to drought conditions can be attributed to its high rate of water use (Watts & Moore 2011). In addition, unlike native riparian plants, it is resilient and able to regenerate immediately after being exposed to fire. Furthermore, it grows approximately 3-4 times faster than native riparian plants and can dominate burned areas within a year after burning (Ambrose & Rundel 2007). The composition of existing native plant communities has no relative effect on the establishment of *A. donax* in riparian habitats, whilst favourable abiotic environmental conditions and vegetative reproduction are more influential (Quinn & Holt 2008).

Factors such as soil temperature, soil moisture, rhizome weight and lack of herbivory can explain the performance of *A. donax* in invaded habitats (Quinn & Holt 2008). Rhizomes are tolerant of a wide range of moisture, soil and nutrient conditions and display rapid photosynthetic rates. Plants are able to rapidly colonise riparian habitats and form dense thickets (Moran & Goolsby 2010). Such features have allowed *A. donax* to form dense monotypic stands in many riparian habitats in Mediterranean climate regions. Furthermore, the abundance of light, water and nutrients in Mediterranean climatic regions is believed to increase its competitive ability (Coffman *et al.* 2004).

1.3.1. The origin and genetic composition of *Arundo donax* populations

In the management of invasive alien species, genetic analysis and molecular studies are an important tool, particularly in determining the native origin(s) of invasive species and providing a greater understanding of invasive population structures (Roderick & Navajas 2004). *Arundo donax* has been considered to be native to North Africa, India, the Middle East, the Mediterranean and the Persian Gulf (Moran & Goolsby 2010; Goolsby *et al.* 2013b), and invasive in North America, Australia, Mexico and South Africa (Moran *et al.* 2011). However, there is great complexity and debate surrounding *A. donax* and its true origin. The complications arise as ancient cultivation has obscured the evolutionary and bio-geographical origin of this ‘cryptogenic species’ (Mariani *et al.* 2010). For example, there is some botanical and historical evidence which suggests that *A. donax* could be of Mediterranean origin, whilst some believe its origins are in east Asia (Hardion *et al.* 2014).

Despite these obscurities, a number of molecular studies have sought to provide clarity on the genetic composition and origins of *A. donax* in various regions. Hardion *et al.* (2014) conducted one of the most comprehensive studies in which the DNA of 127 herbarium samples from the Eurasian region was sequenced to determine the origins of *A. donax*. The study concluded that *A. donax* is likely to have originated in the Middle East. Furthermore, the study suggested that Mediterranean Europe *A. donax* populations which have long been considered as ‘native’ to the region, may actually be from ancient cultivation introductions, and thus not native to the region (Hardion *et al.* 2014).

Additionally, Hardion *et al.* (2014) revealed that *Arundo donax* haplotype diversity was divided into four biogeographic clusters. These are Eastern Himalaya-China (haplotype E), Western Himalaya (haplotype W), Central Himalaya (haplotype C) and Middle East (haplotype M). The Middle East haplotype is made up four entities, namely M1, M2, M3 and M4. The M1

haplotype is common worldwide, particularly in the Mediterranean and in invasive populations including Peru, New Caledonia, and the USA. Furthermore, the M1 haplotype is believed to have ancient origins in the Indus Valley in Pakistan and Afghanistan where the M2, M3 and M4 haplotypes occur (Hardion *et al.* 2014). These findings have supported the notion that Mediterranean populations are ancient introductions originating from the Middle East (Hardion *et al.* 2014).

Invasive *A. donax* populations in South Africa, have recently been found to be of the M1 haplotype, with a lack of genetic diversity (Canavan *et al.* in press). The *A. donax* populations in South Africa are believed to originate from Mediterranean Europe. The Afrikaans common name “Spaanseriet” translates to “Spanish reed” which suggests that it comes from Spain. However, the plant’s exact origins in the Mediterranean or Spain remains unconfirmed (Canavan *et al.* in press). Like South Africa, invasive populations in USA are also of the M1 haplotype, with low genetic variation, and are spread primarily through asexual reproduction (Saltonstall *et al.* 2010; Tarin *et al.* 2013; Hardion *et al.* 2014). However, the origins of these populations have been studied and determined. Most invasions occurring along the Rio Grande river in the USA are believed to originate from Mediterranean Spain as they have the closest genetic proximity to *A. donax* populations from the southern and eastern coast of Spain (Tarin *et al.* 2013). However, an *A. donax* population in Balmorhea, western Texas has unique alleles and is likely to have originated from another Mediterranean region (Goolsby *et al.* 2013a; Tarin *et al.* 2013). In contrast to these invaded regions, *A. donax* invasions in Australia have medium to high genotypic variation in riparian areas, despite reproduction being predominantly asexual (Haddadchi *et al.* 2013).

1.3.2. *Arundo donax* in South Africa

Arundo donax is believed to have been deliberately introduced into South Africa for erosion control in the late 1700’s (Guthrie 2007), and has been identified as one of the many problem plants in South Africa (Bromilow 1995). It is listed as a Category one plant (i.e. declared weed) as per the Conservation of Agricultural Resources Act (Act No. 43 of 1983) and the recent National Environmental Management: Biodiversity Act (Act No. 10 of 2004) of South Africa, meaning that it is prohibited on any water or land surface and it must, where possible, be eradicated or controlled. Furthermore, it has a ‘transformer’ invasive status as per Swarbrick's (1991) categories of environmental weeds suggesting that it is able to replace or dominate canopies or sub-canopies of natural or semi-natural ecosystems and transform the

functioning, integrity and structure of ecosystems. It is commonly referred to as giant reed or Spanish reed and when fully grown can reach heights of 2-6 metres (Henderson 2001). The plant generally spreads from horizontal rootstocks and rarely branches. The leaves grow up to widths of 80 mm and up to lengths of 700 mm and are a pale green to bluish green colour. Unlike the native *Phragmites mauritianus* Kunth. (Poaceae), the leaf tips of *A. donax* are soft or firm and not spiky (Henderson 2001). The cream or brown spear-shaped inflorescence (Fig 1.1.) has a silky texture and is compact, generally remaining between 300 and 600 mm long. Its introduction into South Africa is believed to have been for ornamental purposes, due to its ability to grow to great heights (Bromilow 1995), and it is currently cultivated for screening purposes (Henderson 2001).

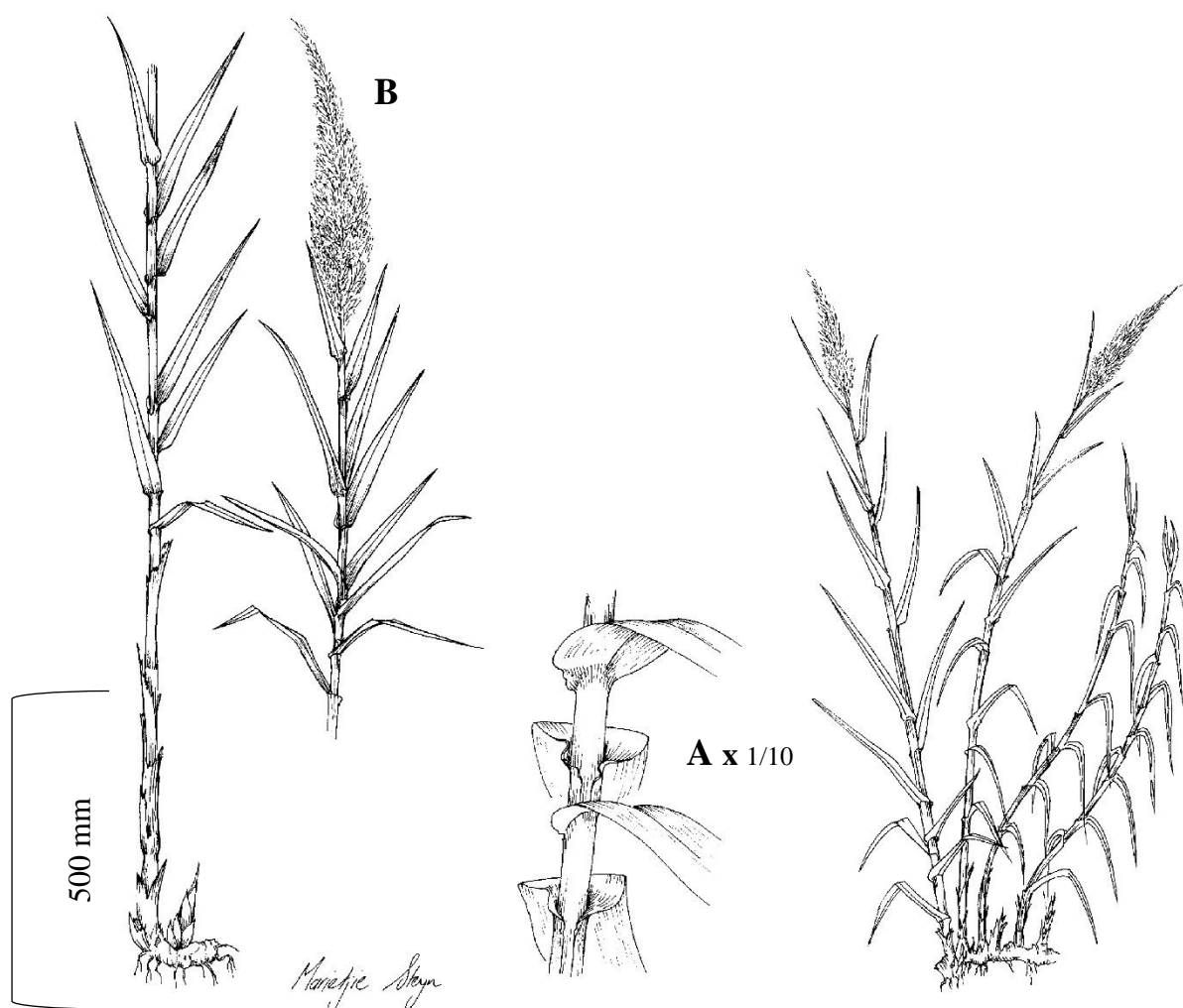


Fig 1.1. *Arundo donax* leaves (A), and inflorescence (B). (Henderson 2001).

Arundo donax invades watercourses but can be found on roadsides and other locations away from water, unlike South Africa's indigenous reeds. Every province in South Africa has been invaded by *A. donax* (Fig 1.2.), with the most abundant and severe invasions occurring in the Western Cape, Gauteng and KwaZulu-Natal. There are mostly light levels of invasion in the Northern Cape and North West which are not widespread. The remaining provinces (i.e. Eastern Cape, Free State, Mpumalanga, Limpopo), are dominated by light to moderate levels of invasion, although there are some locations in these provinces which have high levels of invasion. Coastal areas appear to have been more severely invaded than inland regions. The least frequent occurrences of *A. donax* are found in the Free State and North West Province (Fig 1.2.).

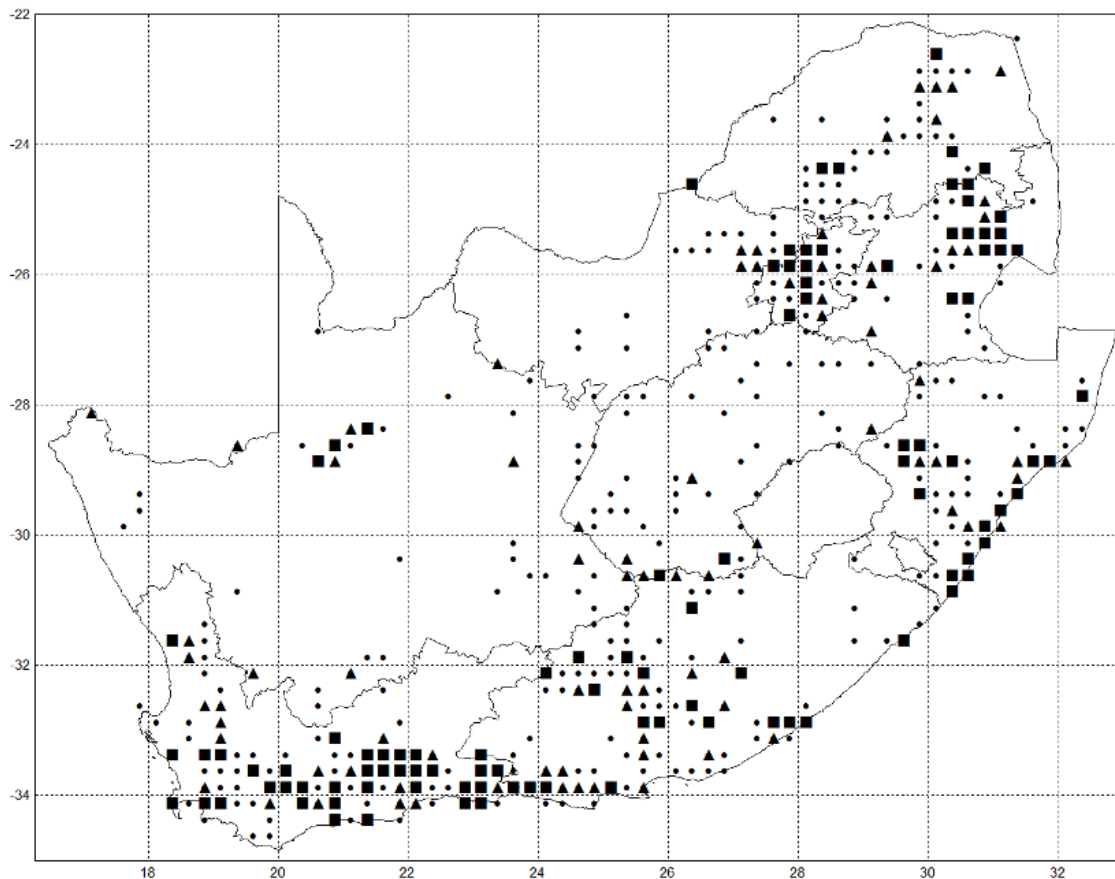


Fig 1.2. Distribution and abundance of *Arundo donax* in South Africa (Drawn by L. Henderson. 2015. Data Source: SAPIA database (Henderson 2011), ARC-PPRI, Pretoria). Dots indicate light levels of invasion, triangles indicate moderate levels of invasion and squares indicate high levels of invasion.

1.3.3. *Arundo donax* in the United States of America

Arundo donax is believed to have initially been introduced into the USA for erosion control, but later also became useful in providing material for thatched roofs and reed musical instruments (Goolsby *et al.* 2009a; Mariani *et al.* 2010; Moran & Goolsby 2010). Since then, 14 of the southern states of the USA have been invaded (Moran & Goolsby 2010), with the most severe invasions occurring along the Rio Grande river in Texas and along the coastal rivers of southern California (Yang *et al.* 2011). Approximately 30 000 hectares of the Lower Rio Grande Basin have been invaded (Moran & Goolsby 2010). Aerial photography along the Rio Grande river on the Texas–Mexico border region recorded 898 kilometres of invaded riparian area, with 38% and 62% on the Mexican and USA sides, respectively (Yang *et al.* 2011).

In Texas, Mexico and California, *A. donax* has become problematic, using scarce water resources which would otherwise have been used by native species. It increases flood risks, provides fuel for fires and inhibits law enforcement (relating to illegal immigration) along the USA-Mexico border (Coffman *et al.* 2004; Goolsby *et al.* 2009b; Moran & Goolsby 2010; Moran 2015). A study conducted on *A. donax* stands along the Lower Rio Grande river in southern Texas found the rate of water use by the plant to be above average (Watts & Moore 2011). *Arundo donax* also degrades the condition of riparian habitats for occupancy by native wildlife (Coffman *et al.* 2004). In southern California, riparian habitats and mature woodlands are at risk due to the *A. donax* invasions, which have threatened the persistence of several native and endemic species. In the absence of control, this invasive reed has the potential to develop into climax communities and alter the structure of natural riparian habitats (Rieger & Kreager 1989). Native riparian plants in the states of California and Florida have been displaced by the reed (Mariani *et al.* 2010; Moran & Goolsby 2010).

1.3.4. Mechanical and chemical control

Bromilow (1995), suggested that achieving control of *A. donax* is very difficult. Mechanical control can only be successful if there is complete biomass removal, particularly the roots, as the plant is very resilient and able to regrow from stems or rhizomes left in the soil. Chemical control can only be successful if stands are first cut down to ground level and then systematically treated with herbicide when they regrow to a height of 1-2 metres. However, optimum and long-term results will only be achieved if thorough follow-up

operations are conducted as needed (Bromilow 1995). In the USA, mechanical and chemical control has involved complete removal of all plant biomass from invaded sites, mulching and cutting, and subsequent treatment of stumps with herbicide (Coffman *et al.* 2004). Such mechanical removal of biomass on an annual basis is unlikely to provide control of *A. donax* stands, as the majority of the plant's biomass is contained below ground. In addition, stems and roots which remain after mechanical biomass removal have been able to regenerate rapidly and produce large clumps (Thornby *et al.* 2007). In addition, the sheer size of the invaded areas, especially those in the arid south-western USA, have rendered chemical and mechanical control operations unfeasible (Mariani *et al.* 2010). Biological control thus appeared to be a more viable, cost effective and long term control option for the USA (Goolsby & Moran 2009; Moran & Goolsby 2010). Cost-benefit analyses and economic impact analyses yielded positive results, which suggested that economic benefits could be gained from a decrease in *A. donax* populations using biological control (Seawright *et al.* 2009).

1.3.5. Biological control

The Agricultural Research Service of the United States Department of Agriculture (USDA-ARS) initiated a research programme on biological control of *A. donax* in an attempt to achieve a more effective control solution. Four herbivorous insects, the Arundo wasp, *Tetramesa romana* Walker (Hymenoptera: Eurytomidae), Arundo scale, *Rhizaspidiotus donacis* (Leonardi) (Hemiptera: Diaspididae), Arundo leafminer, *Lasioptera donacis* Couti and Faivre-Amiot (Diptera: Cecidomyiidae) and Arundo fly, *Cryptonevra* sp. (Diptera: Chloropidae), have been investigated as potential biological control agents for *A. donax* invasions in the USA (Seawright *et al.* 2009). Whilst *T. romana* and *R. donacis* have since been approved and released as biological control agents in the USA (Goolsby *et al.* 2011; Goolsby *et al.* 2014), *L. donacis* is still under pre-release evaluation for safety and efficacy (Goolsby *et al.* 2016), and the Arundo fly, *Cryptonevra* sp. was rejected as it developed on sorghum (Goolsby pers. comm. 2015).

The stem-galling wasp *T. romana* was the first agent to be considered and was confirmed to be specific to the genus *Arundo*, based on no-choice tests conducted in quarantine that suggested that non-target impacts were highly unlikely (Goolsby & Moran 2009). The potential of *T. romana* was reaffirmed by studies revealing its impact on the target plant (Goolsby *et al.* 2009b). In 2009, the wasp was released in Mexico and the lower Rio Grande Basin of Texas, with releases in northern California in 2010 (Goolsby & Moran 2009; Moran

& Goolsby 2010; Moran 2015). However, greater impact on *A. donax* populations was required, highlighting the need for additional agents that targeted different plant parts (Denoth *et al.* 2002; Moran & Goolsby 2010). Impact studies showed that the combined impact of *T. romana* and *R. donacis* (see below) caused significant damage to *A. donax* (Goolsby *et al.* 2009b). Both agents are capable of reducing photosynthesis in different ways, with the effect of *R. donacis* being evident approximately five months after inoculation. In addition, the combined use of both agents has the potential to create a stronger negative physiological effect (Moore *et al.* 2010).

1.4. RHIZASPIDIOTUS DONACIS

Rhizaspidotus donacis is an armoured scale insect that has been established as a biological control agent for *A. donax* in the USA. Armoured scales (Diaspididae) represent a family of around 2400 species (Rosen 1990). The Diaspididae is considered to be the most specialised of all families within the Coccoidea (Rosen 1990), and is commonly found on grasses (Evans 1991). Of the over 250 diaspidid species which are known to occur on grasses (Poaceae), some 58% feed on only one grass genus. Therefore, armoured scales may offer potential biocontrol options for invasive grasses (Moran *et al.* 2011). Furthermore, armoured scales use their maxillary mandibular stylets to pierce parenchyma cells, mesophyll cells, and in certain cases the vascular tissues of roots, stems, leaves and fruit to extract the sap, thereby obtaining nutrients (Rosen 1990; Moran & Goolsby 2010). This sap sucking causes damage to the plant by decreasing its growth (Moran & Goolsby 2010), which can be useful in biological control.

Armoured scale insects are sexually dimorphic. An adult female armoured scale is characterised as being legless, wingless, and having a flattened sac-like body in which the abdomen, thorax and head are fused. In contrast, the abdomen, thorax and head are clearly divided in the body of an adult male and wings, legs and antennae are present (Rosen 1990). An external scale covering envelopes the immobile instars and provides protection from chemical and physical elements and prevents desiccation. The scale cover is formed from waxy secretions of the insect and can therefore be detached without the insect body being damaged (Rosen 1990; Moran & Goolsby 2010).

Rhizaspidiotus donacis follows the typical life cycle of viviparous, bisexual Diaspididae (Moran & Goolsby 2010) (Fig 1.3.) The life cycle of *R. donacis* lasts five to six months (Moran *et al.* 2011), with one generation completed per year (Cortés & Marcos-García 2012). Reproductive females produce minute ‘crawlers’ which represent the first instar stage (Fig 1.3.A). This stage represents the non-feeding, but dispersive and infective, stage as the crawlers are mobile and are responsible for selecting hosts and feeding sites (Koteja 1990; Moran & Goolsby 2010). Temperature plays an important role in this stage and the crawlers are most active between 25 and 32°C. Temperatures above 43 °C are lethal and 13 °C represents their physiological threshold (Koteja 1990). Crawlers are capable of dispersing as far as 150 metres from the parent females. Thereafter, the crawlers settle and begin to feed and moult into second instars (Koteja 1990). Growth continues during the second instar stage and morphological differences between males and females become evident. After the second instar, females develop into reproductive adults (Koteja 1990) (Fig 1.3.B). Adult females can spend three to five months feeding and producing crawlers from the edge of their waxy scale covering (Moran & Goolsby 2010). Second instar males (Fig 1.3.D) undergo two more ‘pupal’ stages before developing into winged adults which emerge from the scale coverings. All instars of the armoured scale’s life cycle are immobile, except for the winged adult males and the crawlers (Rosen 1990). Males are mobile as they are responsible for locating the sessile adult females (Fig 1.3.C) for reproduction (Koteja 1990). In the native range the reproductive stage, which is characterised by adult male emergence, occurs from May to July. Field studies in the native range found crawler emergence, which represent the infective and dispersive stages, to occur during Spring from March to June (Cortés & Marcos-García 2012).

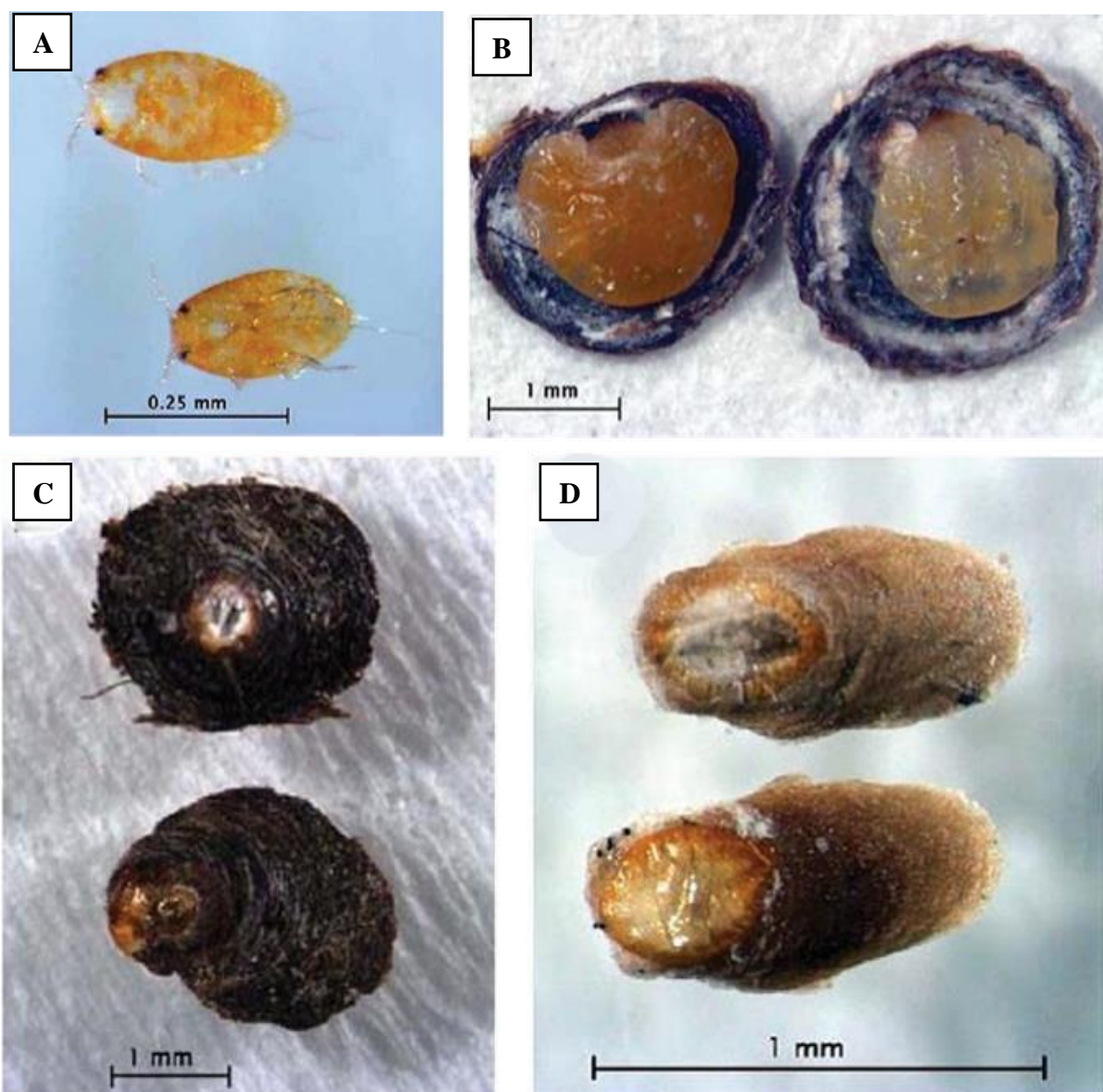


Fig 1.3. Life stages of *Rhizaspidotus donacis*. A – Dorsal and ventral views of crawler; B - Reproductively immature (right) and reproductively mature (left) adult female with scale cover removed; C – Ventral and dorsal views of adult female; D – Ventral and dorsal views of second instar-male with scale covering. (Reproduced from Moran and Goolsby 2010).

1.4.1. *Rhizaspidotus donacis* as a biological control agent

Rhizaspidotus donacis was investigated as a candidate agent due to evidence from collections and literature that suggested its specificity to *A. donax* (Moran *et al.* 2011). The scale was confirmed to be specific to the genus *Arundo* following host-range evaluations in quarantine in the USA and field surveys in the native range. Two USA genotypes of *A. donax*, along with 40 other Poaceae species and five non-grass species were exposed to approximately 200 crawlers

during quarantine no-choice host-specificity testing. Seven ecotypes of *Phragmites australis* (Cav.) Trin. ex Steud., a reed similar to *A. donax*, other closely-related grasses and grasses of economic importance were tested (Goolsby *et al.* 2009a). These test plants were dissected and inspected for crawler survival and development, three months after they were infested. There were normal levels of development on *A. donax* and *A. formosana* Hack. and very low levels of survival on *Leptochloa* species and *Spartina alterniflora* Loisel. Follow-up testing involved the release of 1000 crawlers onto test plants (Goolsby *et al.* 2009a). One adult female was found on *S. alterniflora* and 10 live adult females were found on *Leptochloa virgata* (L.) P. Beauv. The survival on *S. alterniflora* and *L. virgata* was 100 times and 26 times, respectively, lower than survival on *A. donax*. Additional field surveys were conducted in Spain and France where *A. donax* occurred naturally with the two non-target species of concern (Goolsby *et al.* 2009a). In these locations, *R. donacis* was only found on *A. donax*. Furthermore, *R. donacis* was also found to be specific to *A. donax* under field conditions in which potted *Leptochloa* plants and *A. donax* plants were exposed to *R. donacis* for six months. Based on the findings of all these studies, it was suggested that *R. donacis* would be safe for use in biological control and would be unlikely to cause harm to non-target plant species in the USA (Goolsby *et al.* 2009a).

Literature records and collections have suggested that *R. donacis* spans a geographic range which includes southern and eastern Spain, Italy, Crete, southern France, western coastal and southern Turkey and coastal Algeria. The most robust populations appear to be found in Spain (Moran *et al.* 2011). The geographic range, which is rather broad, suggests that *R. donacis* can develop and survive under constant warmth and in cool-warm conditions (Moran & Goolsby 2010). It is also possible that *R. donacis* may occur in India, Nepal, Egypt, Israel, Canary Islands, Morocco, Bulgaria, Sicily, Croatia, Corsica and the Balearic Islands, although no collections have been conducted in these regions (Moran *et al.* 2011). Today, *R. donacis* occurs in Del Rio, Laredo and McAllen in Texas, USA where it has established as a biological control agent (Goolsby pers. comm. 2015). Most *A. donax* genotypes in this area are genetically similar to *A. donax* in Spain, where *R. donacis* is abundant (Moran *et al.* 2011).

In its native range in Mediterranean Europe, *R. donacis* is considered to be the most damaging arthropod on *A. donax* (Goolsby *et al.* 2009a). This scale insect feeds on stems, leaves and rhizomes, allowing several plant parts to be targeted at once (Moran & Goolsby 2010). Studies conducted in the field in the native range and quarantine studies revealed significant impacts on shoot growth, rhizome weight and photosynthetic ability (Moore *et al.*

2010; Cortés *et al.* 2011a; Cortés *et al.* 2011b). Scale infestations substantially reduced the growth rate of shoots in the field in the native range in Spain. The greatest impacts were observed late in Spring which coincided with crawler emergence (Cortés *et al.* 2011a). Field studies in the native range have also revealed a 50% reduction in rhizome weight at sites infested with *R. donacis* relative to uninfested sites (Cortés *et al.* 2011a; Moran *et al.* 2011). Furthermore, quarantine laboratory testing revealed decreased photosynthesis rates as a result of scale infestation (Moore *et al.* 2010).

Several investigations in the USA (see above) confirmed the potential of *R. donacis* as a safe and effective biological control agent, resulting in the first release of the agent in Texas in 2011. Releases were made from *R. donacis* populations originating from genetically similar populations in Alicante, which is on the south eastern coast of Spain (Goolsby *et al.* 2011). So far, there has been evidence of reproductive populations becoming established in the field and evidence of its impact, with lateral shoot deformities of *A. donax* observed at the site of first release (Moran *et al.* 2011). Based on the success of *R. donacis* in the *A. donax* biological control programme in the USA and its damage potential in the native range, this agent is being considered for biological control in South Africa. An important part of this process involves evaluating the suitability of the agent for biological control in South Africa. Some important factors which can influence an agent's suitability and which should be evaluated in pre-release studies are described below and in the chapters to follow.

1.5. FACTORS INFLUENCING SUITABILITY OF AGENTS FOR BIOLOGICAL CONTROL

1.5.1. Climate suitability

Comparing the potential of climate at different locations to support plant or animal populations is often used in pest management, epidemiology and biological control (Sutherst & Maywald 1985). The success of biological control depends on the selection of the correct agent and the selection of an agent that is able to function under the environmental conditions of the area into which it is introduced. The climate, disturbance patterns and resources of the environment of introduction can have a direct effect on the efficacy of a biological control agent (McFadyen 1998). Climate can weaken or elevate the impact of biological control agents

on target plants (Thomson *et al.* 2010; Maines *et al.* 2013; Seastedt 2015). Abiotic environmental variables such as precipitation, humidity and temperature are capable of influencing the establishment and prevalence of biological control agents on target weeds (Dhileepan *et al.* 2013; May & Coetzee 2013; Moran 2015). Likewise, the fitness of both target plants and biological control agents are affected by factors such as moisture, temperature, fire and nutrients (McFadyen 1998). Furthermore, the geographical distribution of many insects is strongly influenced by temperature. The wider the temperature tolerance of an insect agent, the greater its ability to establish in a wide range of climatic conditions (Dhileepan *et al.* 2013). Consequently, climate modelling constitutes an important tool in predicting the establishment and persistence of weed biocontrol agents in their new habitats.

CLIMEX is one of a number of computer-based programmes used to assess the environmental suitability of a new area for a weed or biocontrol agent (Kriticos & Randall 2001; Sutherst *et al.* 2007). The programme allows users to determine how climate is connected to the geographical, seasonal and inter-annual performances of a particular species (Kriticos & Randall 2001). The potential global invasive distribution of *Buddleja davidii* Franchet (Loganiaceae), a weed invasive to New Zealand, Europe and Australia, was modelled using CLIMEX and predicted south-eastern Australia, south-eastern New Zealand, north-eastern Europe, and north-eastern USA to be highly suitable (Kriticos *et al.* 2011). The potential global distribution of *Melaleuca quinquenervia* (Cav.) S.T. Blake (Myrtaceae), an invasive tree which has been introduced to parts of the USA, was also modelled in CLIMEX. The climate model predictions have suggested that there is great potential for further invasion by this tree, especially in south-east Asia, Central America, South America and the Caribbean (Watt *et al.* 2009). Additionally, climate modelling in CLIMEX has contributed to the pre-release suitability testing of a candidate biological control agent, *Cecidochares connexa* (Diptera: Tephritidae), of *Chromolaena odorata* (L.) King and Robinson (Asteraceae) in Australia. According to CLIMEX model predictions, the climatic conditions in regions where *C. odorata* is currently present in Australia, are likely to be climatically suitable for *C. connexa* (Day *et al.* 2016).

1.5.2. Agent survival and impact

Biological control of IAPs comes with several practical and research challenges (Moran *et al.* 2013). In the biological control research process, extensive time and resources are expended on overseas exploration for agents and host-range testing, whilst limited time and

effort are spent on efficacy assessments (Sheppard 2003; McClay & Balciunas 2005; Morin *et al.* 2009). However, pre-release efficacy testing to determine the potential of the candidate agent to control the target plant species is a major component of the research process (Moran *et al.* 2013). Pressure to conduct such pre-release testing to determine which agents have the greatest potential to provide effective control has increased (McClay & Balciunas 2005; Morin *et al.* 2009).

Pre-release efficacy testing is useful in the process of agent selection as it provides an indication of the potential adverse effects of the agent on key growth parameters of the target plant (Sheppard 2003; McClay & Balciunas 2005; Raghu *et al.* 2007; Morin *et al.* 2009). The results from such impact studies, along with those from host-range testing, form the basis of risk assessments, which are used to inform decisions made by regulatory authorities as to whether a candidate agent should be released or not (Moran *et al.* 2013).

Such agent efficacy tests are conducted under controlled conditions in a laboratory or glasshouse or in the field in the native range (Morin *et al.* 2009). The potential efficacy of a candidate agent is determined by assessing the type and extent of its damage to the target weed (Moran *et al.* 2013). For example, the potential impact of *Liothrips tractabilis* Mound and Pereyra (Thysanoptera: Phlaeothripinae) was evaluated as part of the research process that facilitated its release against *Campuloclinium macrocephalum* (Less.) DC. (Asteraceae) in South Africa. The thrips had a significant negative impact on several plant growth parameters including the number of leaves, biomass, plant height and flower production at both medium and high population densities (McConnachie & McKay 2015).

1.6. RESEARCH AIMS AND OBJECTIVES

This study will contribute towards determining the potential efficacy of *R. donacis* as a biological control agent for *A. donax* in South Africa. More specifically, the aims of this study are to: (1) determine the climatic suitability of South Africa to support Spanish *R. donacis* populations; (2) determine if *A. donax* has spread to all suitable areas in South Africa; and (3) determine the suitability, in terms of survival and impact on plant growth, of *R. donacis* from Alicante, Spain for the biological control of *A. donax* in South Africa. These aims will be achieved through the following objectives:

- i. To use climate-modelling software to model the climatic suitability of South Africa for *R. donacis* and *A. donax*.
- ii. To use climate-modelling software to model the climatic similarity of South Africa in relation to regions in the native range of Spain, that are currently supporting *R. donacis* populations.
- iii. To measure the survival of *R. donacis* from Alicante, Spain on the *A. donax* biotype in South Africa.
- iv. To measure the effect of herbivory by *R. donacis* from Alicante, Spain on plant growth parameters of the *A. donax* biotype in South Africa.

CHAPTER 2: THE CLIMATIC SUITABILITY OF SOUTH AFRICA FOR THE INVASIVE REED, *ARUNDO DONAX* AND ITS BIOLOGICAL CONTROL CANDIDATE *RHIZASPIDIOTUS DONACIS*

2.1. INTRODUCTION

Arundo donax (L.) (Poaceae) is believed to have been introduced into South Africa for ornamental purposes (Bromilow 1995) and has since invaded every province, with the most severe infestations occurring in the coastal regions. The perennial reed has invaded the southern regions of the USA and causes several problems including depleting scarce water resources, threatening the persistence of native species (Rieger & Kreager 1989) and providing fuel for fires (Coffman *et al.* 2004; Goolsby *et al.* 2009b; Moran & Goolsby 2010; Moran 2015). The USA has initiated chemical, mechanical (Coffman *et al.* 2004), and biological control of *A. donax* (Goolsby & Moran 2009; Moran & Goolsby 2010). The *Arundo* scale, *Rhizaspidiotus donacis* (Leonardi) (Hemiptera: Diaspididae) is one of the biological control candidates which has been evaluated and released in the USA (Goolsby & Moran 2009; Moran & Goolsby 2010). Native to Mediterranean Europe, this stem-, leaf- and rhizome-feeding scale insect is considered to be the most damaging arthropod of *A. donax* (Goolsby *et al.* 2009a; Moran & Goolsby 2010). *Rhizaspidiotus donacis* is now a candidate biological control agent for *A. donax* in South Africa and pre-release host-range and efficacy testing is being pursued. A key component of this process is an evaluation of the climatic niches of both *A. donax* and *R. donacis* to determine which areas are climatically suitable for the persistence of these species.

The fields of biological control, pest management and epidemiology benefit from predictions of the climatic conditions at different locations to support animal or plant populations (Sutherst & Maywald 1985). In biological control research, climate modelling can aid the prediction of potential weed distributions in invaded regions and biological control agent distributions in proposed regions of introduction. Furthermore, it can determine the climatic similarity between the climate of different locations where a biological control agent is persisting and the climate of a proposed region of introduction. Predicting regions that can support the proliferation of weeds is useful as it makes land and water managers aware of which

regions are at risk of being invaded, allowing surveillance and control efforts to be more focused (Julien *et al.* 1995).

Weed risk assessments attempt to determine the proportion of a region that a weed can possibly persist in and what the associated environmental and economic threats are. Climate modelling can help to determine these factors by modelling the environmental suitability of the area of interest for a weed (Kriticos & Randall 2001). In addition, weed management activities and decision making can benefit from a knowledge of where a biological control agent is likely to survive and be successful (Julien *et al.* 1995; Sutherst *et al.* 2007). Understanding the population phenology of candidate biological control agents in their native ranges is crucial in providing greater insight into the establishment potential of these candidate agents in areas of introduction. Furthermore, it is important to understand how these agents respond to climatic variation, in order to predict how they may respond to such variations in an area targeted for management (Cortés & Marcos-García 2012).

Investigations into the climatic similarities between the native range and regions of introduction of biological control candidates can aid the selection of suitable agents. One of the primary reasons for biological control agents not establishing or developing robust populations is a lack of climatic similarity between the target regions and the area of origin (Robertson *et al.* 2008). Climate matching modelling can help to determine the probability of a biological control agent establishing and persisting in invaded regions (Sutherst *et al.* 2007; Robertson *et al.* 2008). A fundamental requirement for a selected biological control agent to be successful, is an environment in the target region that enables the agent to persist and thrive. Therefore, biological control agents that come from regions with similar climates to that of the invaded region are more likely to be effective in controlling the invasive species (Robertson *et al.* 2008).

The assessments of the climatic suitability of different areas for weeds or biological control agents are often conducted using CLIMEX, which is a computer-based simulation programme well suited to such assessments (Kriticos & Randall 2001; Sutherst *et al.* 2007). The programme allows users to determine how climate is connected to the geographical, seasonal and inter-annual performances of a particular species. This is conducted through three separate modules, namely the “Compare Years”, “Compare Locations” and “Match Climates” modules (Kriticos & Randall 2001). The “Match Climates” module compares long-term meteorological data from the two areas of interest to determine the level of climatic similarity

between them (Sutherst *et al.* 2007). The “Compare Locations” module results in a species-response model (Kriticos & Randall 2001). It is a popular process-oriented module that models the predicted distribution of a species based on the weekly and annual climatic suitability of a number of locations (Kriticos & Randall 2001; Sutherst *et al.* 2007). The module uses long-term meteorological data to describe the response of a species to temperature and moisture and derive weekly and annual population growth indices (Sutherst *et al.* 2007). Species parameters, which represent the species’ responses to the environment, are fitted by using knowledge of the species’ native or introduced distribution in relation to a number of climatic variables (Kriticos & Randall 2001; Sutherst *et al.* 2007).

The climatic suitability of North America for *A. donax* (J. Goolsby unpublished data) and *R. donacis* (Goolsby *et al.* 2013b) was predicted using CLIMEX as part of the USA biological control program, and South Africa is now pursuing a similar study. The aim of this study was to determine the climatic suitability of South Africa to support Spanish *R. donacis* populations and to determine if *A. donax* has spread to all suitable areas in South Africa. The main objectives were therefore to: (1) model the potential distribution of *A. donax* in South Africa using parameters based on its native and introduced range distributions; (2) compare the actual distribution of *A. donax* with the potential distribution as modelled by CLIMEX; (3) model the potential distribution of *R. donacis*, a candidate biological control agent of *A. donax*, in South Africa using parameters based on its native and introduced range distributions; and (4) model the climatic similarity between locations in the Spanish native range of *R. donacis* and those invaded by the weed in South Africa.

2.2. METHODS

Within the CLIMEX modelling system, an estimation of potential population growth and survival in a location requires the use of growth-related indices and stress-related indices. The growth indices describe the potential growth of a population during a favourable season whilst stress indices describe the probability of a population persisting through an unfavourable season. These growth-related and stress-related indices are combined into an Ecoclimatic Index (EI) which characterises the overall climatic suitability of a given location for the species of interest (Sutherst *et al.* 2007). The EI ranges between zero and 100 with EI values of zero indicative of the growth and stress requirements not being met and the location being

unfavourable for survival. EI values below 10 are indicative of long-term survival being unlikely at the location due to the lack of climatic favourability. Locations which are moderately favourable for the survival of a population are characterised by EI values between 10 and 30, whilst locations with values above 30 are considered to be highly favourable for supporting substantial populations (Sutherst *et al.* 2007). The “Match Climates” module calculates a Composite Match Index (CMI) which provides an indication of the level of climatic similarity between the two locations and CMI values above 0.5 are considered to reflect closer climate similarity (Sutherst *et al.* 2007).

2.2.1. Climate modelling

The “Compare Locations” module in CLIMEX was used to model the predicted distribution of *A. donax* and *R. donacis* in South Africa based on their native range distributions. A set of parameters that had already been developed, and that were based on the native range of *A. donax* in Europe and of *R. donacis* in Spain, France and Portugal, were obtained from Goolsby (unpublished data) and Goolsby *et al.* (2013b), respectively (Table 2.1.). The parameters for *A. donax* did not use light index, diapause index, cold-dry stress, cold-wet stress, hot-dry stress, whilst the *R. donacis* parameters did not use these indices and hot-wet stress (Table 2.1.). These parameters were excluded either due to irrelevance to the modelled species or lack of information to sufficiently adjust them. The models were run without climate change or irrigation scenarios, using the station data simulation.

Table 2.1. CLIMEX parameters for *A. donax* (J. Goolsby unpublished data) and *R. donacis* (Goolsby *et al.* 2013b) based on their native range distributions.

Parameters	Description	<i>A. donax</i>	<i>R. donacis</i>
<i>Moisture Index</i>			
SM0	Limiting low moisture index	0.1	0.1
SM1	Lower optimal moisture index	0.4	0.4
SM2	Upper optimal moisture index	0.7	0.7
SM3	Limiting high moisture index	1.5	1.5
<i>Temperature Index</i>			
DV0	Limiting low temperature (°C)	10	10
DV1	Lower optimal temperature (°C)	20	20

DV2	Upper optimal temperature (°C)	24	24
DV3	Limiting high temperature (°C)	28	28
PDD	Minimum degree-days above DV0 (DD)	810	810
<i>Cold Stress</i>			
TTCS	Cold stress temperature threshold (°C)	2.5	3
THCS	Cold stress temperature rate	0	0
DTCS	Cold stress degree-day threshold (DD)	5	12
DHCS	Cold stress degree-day rate	-0.0011	-0.001
TTCSA	Cold stress temperature threshold (Average) (°C)	0	0
THCSA	Cold stress temperature rate (Average)	0	0
<i>Heat Stress</i>			
TTHS	Heat stress temperature threshold (°C)	30	30
THHS	Heat stress temperature rate	0.002	0.002
DTHS	Heat stress degree-day threshold (DD)	0	0
DHHS	Heat stress degree-day rate	0	0
<i>Dry Stress</i>			
SMDS	Dry stress threshold	0.02	0.02
HDS	Dry stress rate	-0.05	-0.05
<i>Wet stress</i>			
SMWS	Wet stress threshold	1.6	1.6
HWS	Wet stress rate	0.0015	0.0015
<i>Hot-Wet Stress</i>			
TTHW	Hot-Wet temperature threshold (°C)	23	-
MTHW	Hot-Wet moisture threshold (°C)	0.5	-
PHW	Hot-Wet stress rate	0.075	-
<i>Day-degree accumulation</i>			
MTS	Time step of the CLIMEX model	7	7
DVCS	Threshold temperature for calculation of degree-day based heat stress (°C)	10	10
DVHS	Threshold temperature for calculation of degree-day based cold stress (°C)	28	28

The *A. donax* model was then revised using parameters obtained from Barney & DiTomaso (2011) (Appendix I), which were based on native and introduced range distributions. These parameters were revised by fitting them to the known distributions in the introduced range of the USA (EDDMapS 2015). The parameters were iteratively adjusted until there was sufficient visual match to the distribution records. These revised parameters of *A. donax* did not use light index, diapause index, wet stress, cold-dry stress, cold-wet stress and hot-dry stress and were run without climate change or irrigation scenarios (Table 2.2.). As in the previous model, these parameters were excluded either due to irrelevance to the modelled species or lack of information to sufficiently adjust them. During the fitting process, the grid data simulation was used to aid interpolation at all locations, whilst the station data simulation was used when modelling the predicted distribution in South Africa based on these fitted parameters. During the fitting process, there were several outliers which fell outside of the model's predictions. Further investigation found these outliers to occur in cultivation or riparian areas where they would receive additional water inputs. The effect of these conditions could be simulated through the inclusion of an irrigation scenario in the model. In this study, the irrigation scenario was not used for modelling predicted distributions in South Africa.

Likewise, the *R. donacis* model was revised using the parameters from Goolsby *et al.* (2013b), which were based on native range distributions of the scale. The parameters were iteratively adjusted until there was sufficient visual match to three locations in the USA, namely Del Rio, Laredo and McAllen, where *R. donacis* has established as a biological control agent (Goolsby pers. comm. 2015). These revised parameters of *R. donacis* did not use light index, diapause index, cold-dry stress, cold-wet stress, hot-dry stress and hot-wet stress and were run without climate change or irrigation scenarios (Table 2.2.). During the fitting process, the grid data simulation was used to aid interpolation at all locations, whilst the station data simulation was used when modelling the predicted distribution in South Africa based on these fitted parameters.

Table 2.2. CLIMEX parameters for *A. donax* and *R. donacis* based on their native and introduced range distributions.

Parameters	Description	<i>A. donax</i>	<i>R. donacis</i>
<i>Moisture Index</i>			
SM0	Limiting low moisture index	0.1	0.1
SM1	Lower optimal moisture index	0.2	0.4

SM2	Upper optimal moisture index	2	0.7
SM3	Limiting high moisture index	10	1
<i>Temperature</i>			
<i>Index</i>			
DV0	Limiting low temperature (°C)	10	10
DV1	Lower optimal temperature (°C)	20	20
DV2	Upper optimal temperature (°C)	35	28
DV3	Limiting high temperature (°C)	40	38
PDD	Minimum degree-days above DV0 (DD)	810	810
<i>Cold Stress</i>			
TTCS	Cold stress temperature threshold (°C)	2.5	3
THCS	Cold stress temperature rate	0	0
DTCS	Cold stress degree-day threshold (DD)	5	12
DHCS	Cold stress degree-day rate	-0.0011	-0.001
TTCSA	Cold stress temperature threshold (Average) (°C)	0	0
THCSA	Cold stress temperature rate (Average)	0	0
<i>Heat Stress</i>			
TTHS	Heat stress temperature threshold (°C)	40	38
THHS	Heat stress temperature rate	0.002	0.002
DTHS	Heat stress degree-day threshold (DD)	0	0
DHHS	Heat stress degree-day rate	0	0
<i>Dry Stress</i>			
SMDS	Dry stress threshold	0.01	0.04
HDS	Dry stress rate	-0.005	-0.05
<i>Wet stress</i>			
SMWS	Wet stress threshold	1.6	1.6
HWS	Wet stress rate	0.0015	0.0015
<i>Hot-Wet Stress</i>			
TTHW	Hot-Wet temperature threshold (°C)	35	-
MTHW	Hot-Wet moisture threshold (°C)	1	-
PHW	Hot-Wet stress rate	0.075	-

<i>Day-degree</i>			
<i>accumulation</i>			
MTS	Time step of the CLIMEX model	7	7
DVCS	Threshold temperature for calculation of degree-day based heat stress (°C)	10	10
DVHS	Threshold temperature for calculation of degree-day based cold stress (°C)	28	28

The “Match Climates” module was used to determine the climatic similarity between locations in the native range of *R. donacis* and those in South Africa. The locations selected for use as ‘home’ locations were Barcelona and Valencia which are both located in Spain, whilst Africa was selected as the ‘away’ location. No climate change or irrigation scenarios were used. An equal weighting of one was used for all temperature and rainfall indices, except for relative humidity and soil moisture which had a weighting of zero. This allowed for temperature and rainfall patterns to be driving variable in identifying locations with similar climate patterns.

2.2.2. GIS mapping

ArcMap Version 10.2 was used for creating the final map outputs reflecting the provinces and key localities in South Africa, predicted distributions and climate similarity. Shapefiles used for mapping were sourced from the Geography Department of the University of KwaZulu-Natal (UKZN), Pietermaritzburg. For the maps generated from the “Compare Locations” modelling data, EI values below 9 were classified as unfavourable, those below 30 were classified as suitable for moderate population growth, and those of 30 and above were classified as highly suitable for substantial population growth. The actual *A. donax* distribution in South Africa (Data Source: SAPIA database (Henderson 2011), ARC-PPRI, Pretoria) was added to the “Compare locations” map outputs for ease of comparison. For maps generated from the “Match Climates” modelling data, CMI values below 0.6, between 0.6 and 0.7 and above 0.7 were classified as indicative of low levels of climate similarity, moderate climate similarity and high climate suitability, respectively. Additionally, a map has been provided to indicate the different provinces of South Africa and key localities (Appendix II).

2.3. RESULTS

2.3.1. Climatic suitability of South Africa for *Arundo donax*

According to the CLIMEX model predictions, using parameters based on *A. donax*'s native range distribution alone, the Free State, Gauteng, Limpopo and Mpumalanga, inland regions of KwaZulu-Natal and coastal and inland regions of the Western Cape and Eastern Cape are favourable for invasion by *A. donax*. The highest EI values were predicted for coastal locations along the Western Cape and Eastern Cape coast and the highest number of favourable locations were predicted for the same provinces (Fig 2.1.). Unfavourable areas with EI values below 10 were predicted throughout the Northern Cape and North West (Fig 2.1.). Additionally, there is limited matching between the model predictions and the actual *A. donax* distributions in the country (Fig 2.1.).

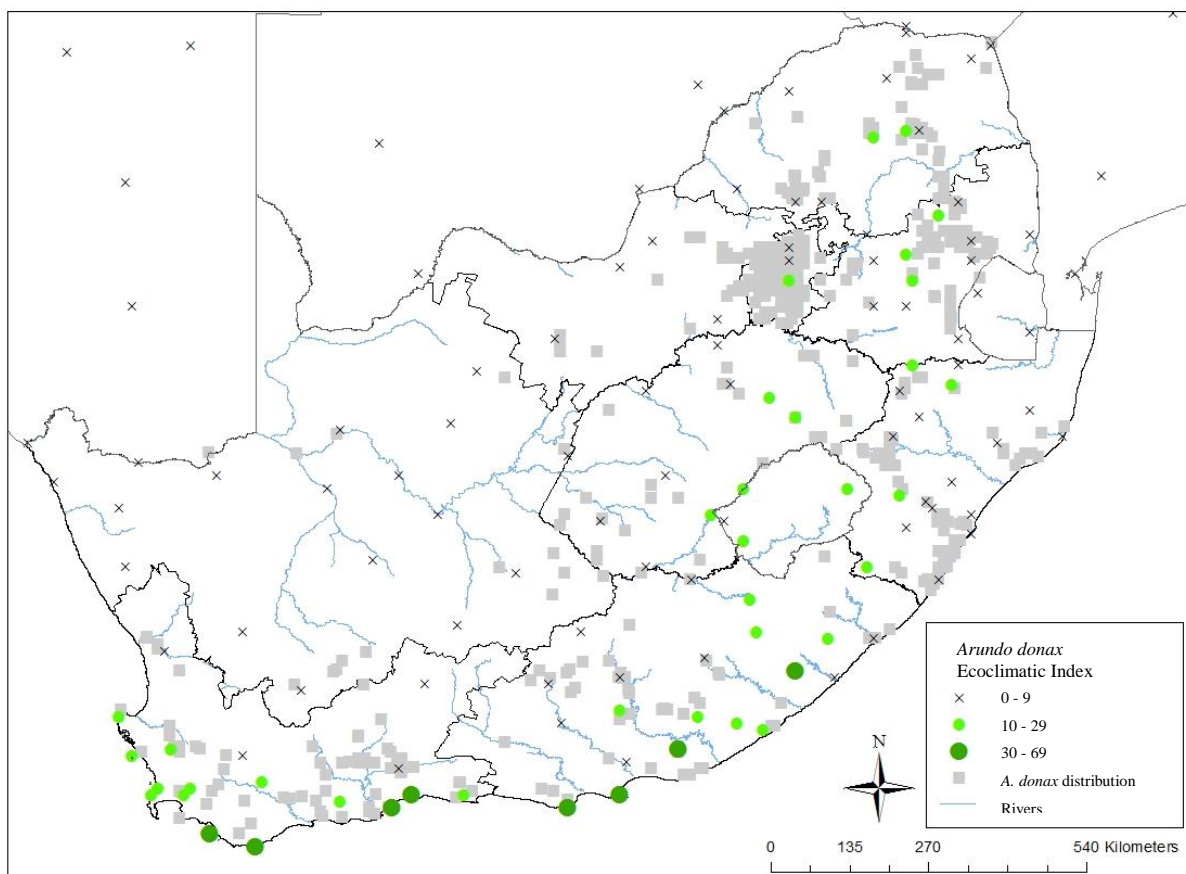


Fig 2.1. Climatic suitability of South Africa for *A. donax* using parameters based on its native range distribution, in comparison to the actual *A. donax* distribution in South Africa (Data Source: SAPIA database (Henderson 2011), ARC-PPRI, Pretoria).

According to the CLIMEX model predictions based on parameters fitted to both the native and introduced range distributions, every South African province has areas which are climatically favourable for *A. donax*. The highest EI values were predicted for coastal locations in KwaZulu-Natal and the Eastern Cape and the highest numbers of favourable locations were predicted for the same provinces (Fig 2.2.). Unfavourable areas with EI values below 10 were predicted throughout the Northern Cape and in a minimal number of locations in the Western Cape, Eastern Cape and Free State. Lower but favourable EI values were predicted in the western Free State, north-western Eastern Cape, south-western Western Cape and eastern Northern Cape (Fig 2.2). In contrast to model predictions based on the native range alone (Fig 2.1.), the revised model predictions have a greater match with actual *A. donax* distributions in the country (Fig 2.2.).

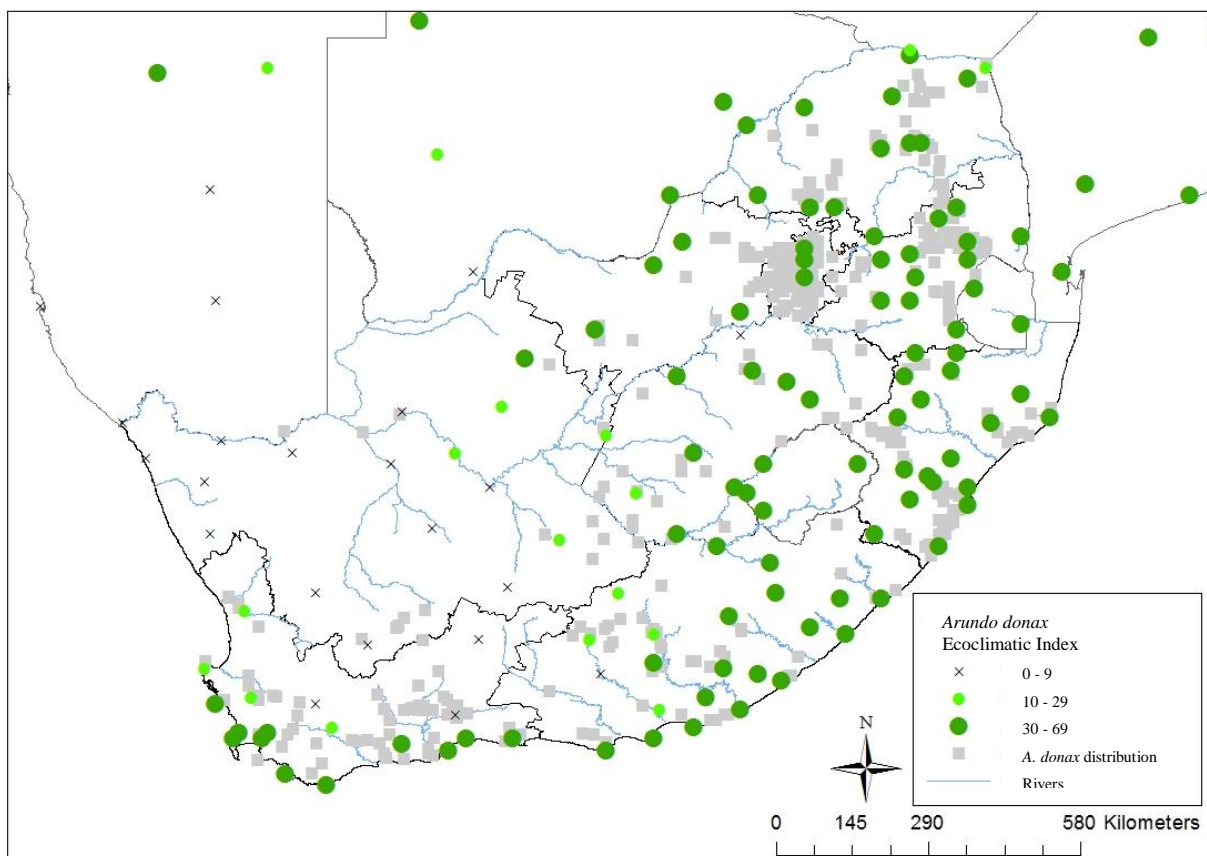


Fig 2.2. Climatic suitability of South Africa for *A. donax* using parameters based on both its native range and introduced range distributions, in comparison to the actual *A. donax* distribution in South Africa (Data Source: SAPIA database (Henderson 2011), ARC-PPRI, Pretoria).

2.3.2. Climatic suitability of South Africa for *Rhizaspidiotus donacis*

According to the CLIMEX model predictions, using parameters based on its native range distribution alone, the inland provinces of the Free State, Gauteng, Limpopo and Mpumalanga, and the coastal provinces of KwaZulu-Natal, Western Cape and Eastern Cape are favourable for the survival of *R. donacis*. The highest EI values were predicted for coastal (but also some inland) locations in the Western Cape, Eastern Cape and KwaZulu-Natal, with the highest numbers of favourable locations predicted for the same provinces (Fig 2.3.). Unfavourable areas with EI values below 10 were predicted throughout the Northern Cape and North West provinces, and throughout most of the Free State province (Fig 2.3.).

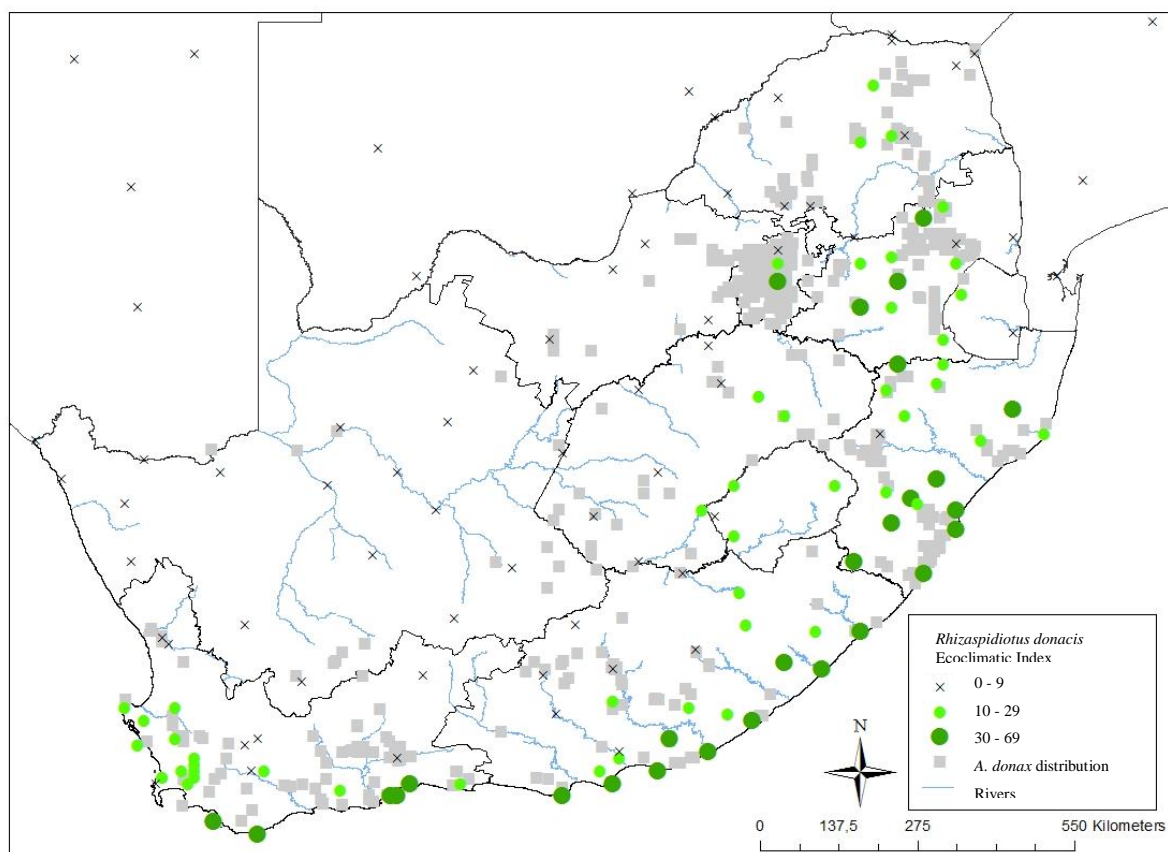


Fig 2.3. Climatic suitability of South Africa for *R. donacis* using parameters based on its native range distribution, in relation to the actual distribution of *A. donax* (Data Source: SAPIA database (Henderson 2011), ARC-PPRI, Pretoria).

According to the CLIMEX model predictions based on parameters fitted to both native and introduced range distributions, every South African province has areas that are climatically favourable for *R. donacis*, although this included only one suitable location in the Northern

Cape. The highest EI values were predicted for coastal locations in the Eastern Cape and inland and coastal locations in KwaZulu-Natal and the highest numbers of favourable locations were predicted for the same provinces (Fig 2.4.). Unfavourable areas with EI values below 10 were predicted throughout the Northern Cape and in a minimal number of locations in the Western Cape and Eastern Cape. Lower but favourable EI values were predicted in every province (Fig 2.4.).

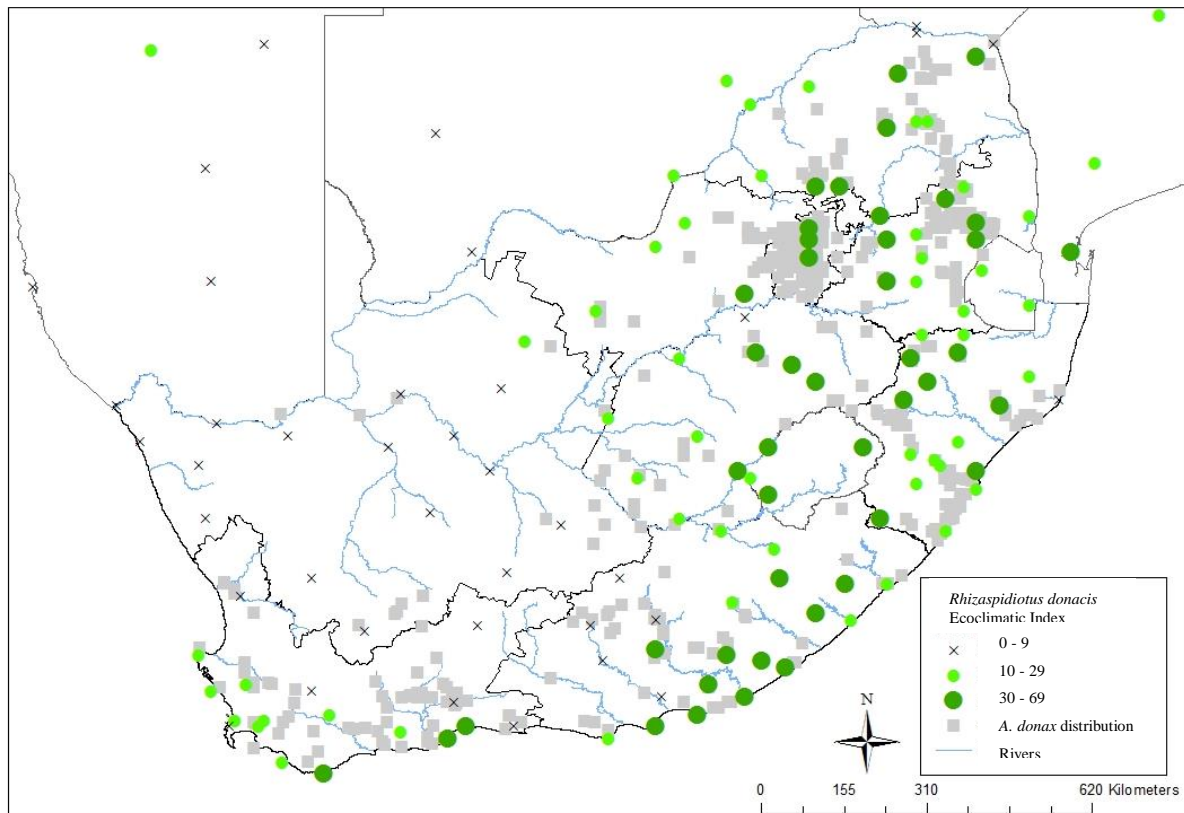


Fig 2.4. Climatic suitability of South Africa for *R. donacis* using parameters based on its native and introduced range distributions, in relation to the actual distribution of *A. donax* (Data Source: SAPIA database (Henderson 2011), ARC-PPRI, Pretoria).

2.3.3. Climate matching

The CLIMEX “Match Climates” model that compared Barcelona and Valencia with South Africa predicted climate similarity throughout the country. The greatest number of locations in South Africa with CMI values above 0.7 in relation to the climate of Barcelona (Fig 2.5.A), are predicted for the Eastern Cape and Western Cape, with the Free State following closely behind. The greatest numbers of locations with CMI values above 0.7 in relation to the

climate of Valencia (Fig 2.5.B) are predicted for the Eastern Cape, Western Cape and Northern Cape with the Free State following closely behind. The highest CMI values for climate matching with Barcelona and Valencia were predicted to be 0.85 and 0.89, respectively. Lower levels of climatic similarity (i.e. CMI below 0.6) with Barcelona were predicted for the Northern Cape, Limpopo, Mpumalanga and KwaZulu-Natal, whilst lower levels of climatic similarity with Valencia were predicted for Limpopo, Mpumalanga, Gauteng and KwaZulu-Natal (Fig 2.5.).

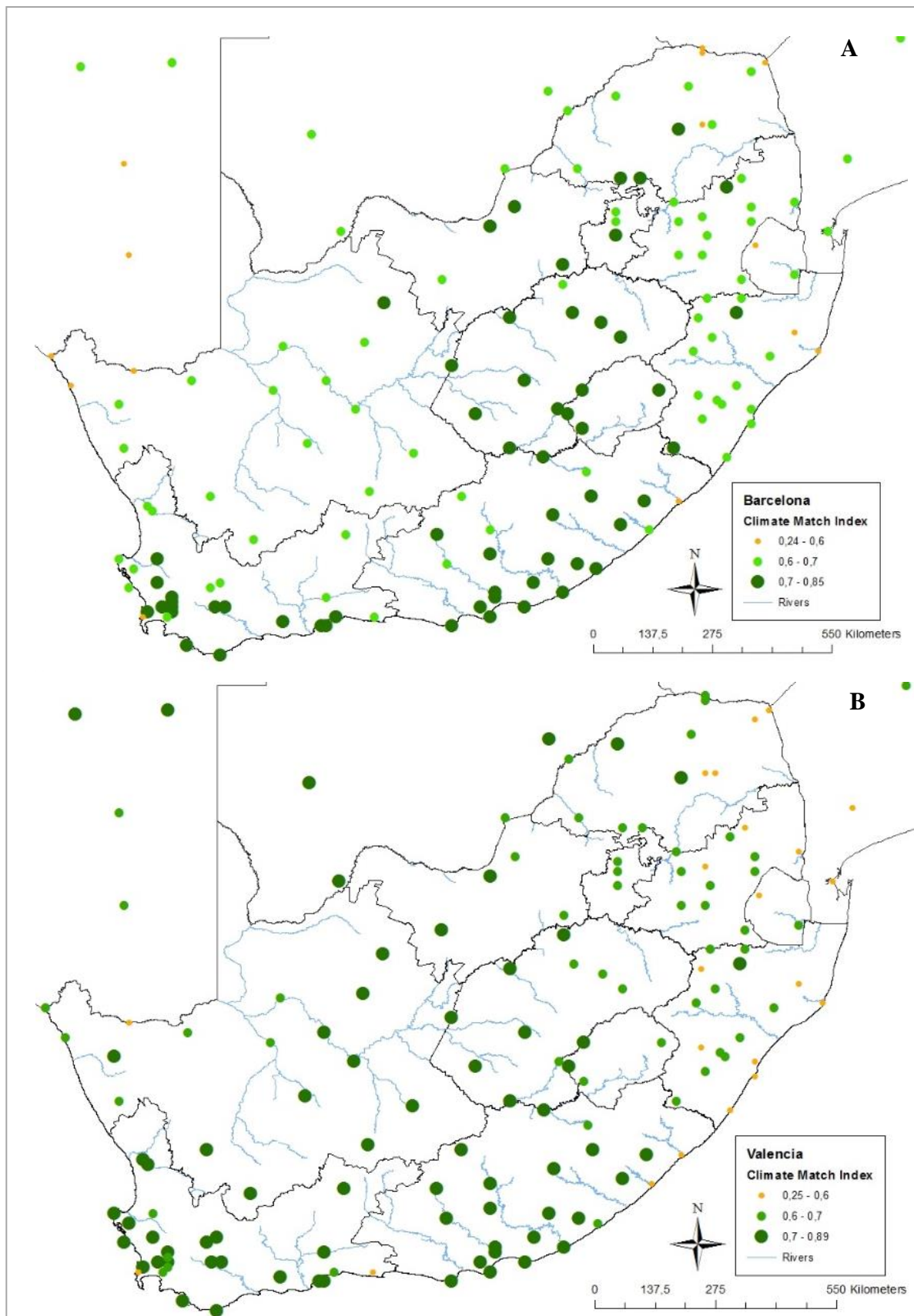


Fig 2.5. Climatic similarity between (A) Barcelona, Spain and (B) Valencia, Spain and South Africa.

2.4. DISCUSSION

In this study, predictions of *A. donax* distributions were made using native range distribution data alone, and both native and introduced range distribution data combined. The model results showed a greater degree of accuracy in predictions modelled with native and introduced range data, than with native range data alone. The predictions that included introduced range data had a greater degree of overlap with actual *A. donax* distribution records, compared to predictions based only on native range data. This demonstrates that predictions of species distributions can be substantially improved by including both native and introduced range parameters. Similarly, Shabani & Kumar (2015) demonstrated that the use of native range data or introduced range data alone can limit the predictions of climate models. McConnachie *et al.* (2011) revised the CLIMEX model for *Parthenium hysterophorus* L. (Asteraceae) using native and introduced range distribution data, and thereby provided more comprehensive predictions of potential distributions in eastern and southern Africa. Broennimann & Guisan (2008) also suggested that models based on native range distribution data alone can fail to predict the full extent of biological invasions. These authors obtained improved predictions of the invasion extent of *Centaurea maculosa* Lam. (Asteraceae), spotted knapweed, in North America, by using introduced range distribution data, instead of European native range data alone (Broennimann & Guisan 2008).

The models predicted most areas along coastal South Africa and parts of the interior with humid subtropical, marine west coastal, Mediterranean and semi-arid climates to be suitable for *A. donax*. These areas represent the same climates in which *A. donax* persists in its native and introduced ranges. The Western Cape climate has the same Mediterranean climatic conditions (Gabler *et al.* 2009) as the European native range of *A. donax* (Cortés & Marcos-García 2012), and as the invasive range of *A. donax* in California (Ambrose & Rundel 2007). Similarly, much of Mediterranean Europe was predicted to be climatically suitable for *A. donax* using climate modelling (Barney & DiTomaso 2011). The coastal regions of South Africa have a humid subtropical climate which is similar to the climate in the regions of eastern Texas that are invaded by *A. donax*. The semi-arid interior regions of South Africa, which were predicted to be climatically suitable, are similar to semi-arid regions in central Texas (Gabler *et al.* 2009), where *A. donax* is invasive (Tracy & DeLoach 1998; Cortés & Marcos-García 2012). Similarly, many semi-arid parts of the southern USA were predicted to be climatically suitable for *A. donax* using climate modelling (Barney & DiTomaso 2011). Adaptation to arid regions was

supported by Mann *et al.* (2013) who demonstrated great drought tolerance in mature *A. donax* plants. Additionally, there is evidence that *A. donax* ecotypes from arid areas are more adapted to drought stress in terms of water movement through xylem vessels, whilst those from sub-Mediterranean habitats are more susceptible to drought stress (Haworth *et al.* 2015). These findings suggest that *A. donax* is able to adapt to the climate and abiotic stresses of both arid and sub-Mediterranean habitats (Haworth *et al.* 2015), which is confirmed by known distributions of *A. donax* in South Africa, USA and the native Mediterranean range.

Some arid areas in the Northern Cape were predicted to be unsuitable despite known distributions of *A. donax*. It is possible that these areas were predicted to be unsuitable due to insufficient annual rainfall. However, the distributions of *A. donax* at these locations are close to or along riparian habitats where they receive additional water. *Arundo donax* is often found growing in riparian zones, and it is possible that the higher soil moisture allows them to successfully invade these areas (Haworth *et al.* 2015). Similarly, many of the infestations in the arid regions of the USA occur along the Rio Grande river or in riparian areas (Goolsby *et al.* 2009a). The occurrence of most *A. donax* infestations in arid areas occurring in close proximity to riparian areas suggest that soil moisture is an important limiting factor to *A. donax* distributions. In addition, cold stress is a limiting factor for *A. donax* distributions (Pompeiano *et al.* 2015). Whilst *A. donax* performs best in warmer areas, it has a lack of cold tolerance which can limit its spread into colder areas. Under mild winters, *A. donax* can continually produce and grow new shoots from rhizomes which are quite cold resistant, but extended exposure to cold conditions (i.e. $< 0^{\circ}\text{C}$) can cause substantial damage to the plant (Pompeiano *et al.* 2015).

Locations in the Western Cape, which have a Mediterranean climate (Gabler *et al.* 2009), were predicted to be suitable for *R. donacis*. These predictions are in agreement with known native distributions of *R. donacis* in the warmer Mediterranean regions of Spain, Greece, France, Italy and Portugal (Goolsby *et al.* 2013b). Similarly, a CLIMEX model with parameters based on native range *R. donacis* distributions predicted California, which has a warm Mediterranean climate (Gabler *et al.* 2009), to be climatically suitable for *R. donacis* (Goolsby *et al.* 2013b) and the region is widely invaded by *A. donax* (Ambrose & Rundel 2007). In addition, humid subtropical, marine west coastal and semi-arid steppe climates, which occur along the east coast and interior of South Africa (Gabler *et al.* 2009) were predicted to be most suitable for *R. donacis*. These climate types do not occur in the European native range of *R. donacis*, although humid subtropical and semi-arid climates do occur in the

parts of Texas (Gabler *et al.* 2009), where *R. donacis* has successfully established as a biological control agent (Goolsby pers. comm. 2015). Similar to South Africa, humid subtropical and semi-arid parts of Texas (Gabler *et al.* 2009) were predicted by climate modelling to be climatically suitable for *R. donacis* (Goolsby *et al.* 2013b).

The arid desert regions of South Africa which are found in parts of the Northern Cape and the North West provinces, were predicted to be unsuitable for *R. donacis*. This is in agreement with model predictions in the USA, in which the desert climates of Arizona were predicted to be unsuitable (Goolsby *et al.* 2013b). It is likely that the arid regions are too dry to support *R. donacis* populations. Rainfall and soil moisture is important for *R. donacis* survival as it stimulates new shoot growth in *A. donax* which become a primary settling location for crawlers (Goolsby *et al.* 2013b). Therefore, a lack of suitable settling sites could hinder the persistence of *R. donacis* populations. While Texas does not experience predictable rainfall at the time of crawler emergence, rhizomes have continuous access to moisture as they mostly grow in riparian areas where they extend down to the water table. Therefore, Goolsby *et al.* (2013b) suggested that despite the lack of rainfall, *A. donax* plants in Texas will still be able to support *R. donacis* populations. Thus far, *R. donacis* has successfully established in these regions (Goolsby pers. comm. 2015), confirming that the conditions are suitable to support *R. donacis*. Similarly, most of the *A. donax* infestations in the arid regions of South Africa occur along riparian areas where they have continual access to moisture. Therefore, it is likely that *A. donax* plants which occur in riparian habitats of arid regions will be able to support *R. donacis* populations.

Climate matching modelling was used to predict where *Hypocosmia pyrochroma* Jones (Lepidoptera: Pyralidae) was most likely to establish in Australia and South Africa, as a biological control agent for *Dolichandra unguis-cati* (L.) Lohman (Bignoniaceae), commonly known as cat's claw creeper. The model predicted that climates in the coastal regions of Australia and South Africa were more favourable than inland regions (Dhileepan *et al.* 2013). Likewise, climate matching was used to determine areas worldwide which matched the climatic conditions of the native range of the alligator weed flea beetle, *Agasicles hygrophila* Selman and Vogt (Coleoptera: Chrysomelidae), the biological control agent of alligator weed, *Alternanthera philoxeroides* (Mart.) Griseb. (Amaranthaceae). Parts of Africa, southern and eastern Australia and southern Europe were predicted to be suitable (Julien *et al.* 1995). In this study, the predictions of the "Match Climates" model were similar to those of the "Compare Locations" model for *R. donacis*, suggesting that most regions in South Africa have a similar

climate to that of Barcelona and Valencia in the native range of *R. donacis* in Spain. However, the “Match Climates” predictions cover more extensive regions of South Africa than the “Compare Locations” predictions for *R. donacis*. In addition, the model predicted that some arid desert regions of the Northern Cape and North West provinces will have low climatic similarity to the native range locations, which is in contrast to the predictions of the “Compare Locations” model. These results should be interpreted with caution due to the simplistic nature of the “Match Climates” module (Sutherst *et al.* 2007). This analysis is the most limited of all CLIMEX modules as it only finds matches between climate patterns (Kriticos & Randall 2001). The “Match Climates” module compares long-term meteorological data from two areas of interest to determine the level of climatic similarity between them and is more useful when there is limited information on the current distribution of a species (Kriticos & Randall 2001; Sutherst *et al.* 2007).

In conclusion, it appears that *A. donax* has occupied a large proportion of potentially suitable sites in South Africa (i.e. Mediterranean, humid subtropical, marine west coastal and semi-arid regions). However, the persistence of *A. donax* in riparian areas which were predicted to be unsuitable due to their aridity, suggests that spread into ‘unsuitable’ arid areas can still occur. Therefore, riparian habitats in arid areas are still at risk of being invaded and should be closely monitored. Furthermore, South Africa appears to be climatically suitable for the establishment of *R. donacis* in the same climatic regions which are suitable for *A. donax*. Although arid areas are typically unsuitable for *R. donacis*, *A. donax* plants may still be able to support *R. donacis* establishment if they have continuous access to water. Therefore, the typical South African climate seems likely to support the biological control of *A. donax* using *R. donacis*.

CHAPTER 3: A PRE-RELEASE EVALUATION OF THE SURVIVAL AND IMPACT OF *RHIZASPIDIOTUS DONACIS* OF SPANISH ORIGIN ON *ARUNDO DONAX* IN SOUTH AFRICA

3.1. INTRODUCTION

Pre-release efficacy testing forms an important component of the biological control research process and helps to determine the suitability of a candidate biological control agent for release (Moran *et al.* 2013). In order to decrease the likelihood of an ineffective biological control agent being released, it is important to prioritise and select agents that demonstrate high levels of damage to the target weed (McClay & Balciunas 2005; Goolsby *et al.* 2009b). The release of ineffective biological control agents wastes resources and can create a negative perception of biological control. In addition, the selection of ineffective agents can yield ecological and economic costs which can be avoided if sufficient pre-release efficacy testing is conducted (McClay & Balciunas 2005).

The efficacy of biological control is often dependent on the origin and genetic variability of the agent and the invasive weed populations (Estoup & Guillemaud 2010). In biological control, agent-weed mismatching can result in failed establishment of released agents and in turn, resource wastage (McFadyen 2003). It is most effective, where possible, to choose agents which originate from the same geographical region as the source population of the target weed (Estoup & Guillemaud 2010). The increased effectiveness of agents from areas of weed origin may be due to coevolution with, and possible local adaptation to, the host plant species (Roderick & Navajas 2003; Estoup & Guillemaud 2010). Pre-release efficacy testing can assess the suitability of candidate agents for the biological control of target weed biotypes found in the invaded range by evaluating factors such as survival, establishment, fecundity and reproduction of candidate agents on the target plants (Goolsby *et al.* 2013a).

Not all biological control agents which establish and reach high densities have an impact on IAP populations (Myers 2000; McClay & Balciunas 2005). Biological control programmes can thus benefit from pre-release impact assessments that determine the probable level of impact of a biological control agent on a target weed so as to select and prioritise the

most effective agents (Goolsby *et al.* 2004b; Goolsby *et al.* 2009b). The process provides an indication of the adverse effects of a candidate agent on key growth parameters of the target plant (Sheppard 2003; McClay & Balciunas 2005; Raghu *et al.* 2007; Morin *et al.* 2009). Pre-release impact assessments aid the determination of per-capita effects which is essentially a measurement of the effect of exposure to known population densities of a biological control agent on plant performance parameters (McClay & Balciunas 2005). Some of the performance parameters assessed include competitive ability, biomass and seed production (McClay & Balciunas 2005; Bownes *et al.* 2010). Although effects on individual plants cannot be translated directly into population effects, the information obtained is still useful. For example, one can determine that if an agent does not cause substantial damage at an individual plant level, it is unlikely to have a substantial effect at a population level (McClay & Balciunas 2005). Additionally, such assessments provide greater understanding of the relationship between agent density and plant size or survival and thereby allow inferences to be made about an agent's potential to suppress populations of the target plant (Sheppard & Smyth 2001; Sheppard *et al.* 2003). If this information is combined with target weed population studies at different sites, ecological models of agent-weed interactions can then be developed (Sheppard *et al.* 2003).

Arundo donax, or Giant reed, is a highly invasive perennial reed in the USA, Australia, Mexico and South Africa (Moran *et al.* 2011). *Rhizaspidiotus donacis*, or the Arundo scale insect, from eastern Spanish collections was released and became established in the USA for the biological control of *A. donax* (Goolsby *et al.* 2011; Moran 2015). Armoured scale insects have the potential to become locally adapted to individual host genotypes because of their close relationships with hosts, which develop due to their immobile nature (Morse & Normark 2006). *Rhizaspidiotus donacis* from Alicante, Spain developed on all three of the *A. donax* biotypes tested in the USA. The closely-matched *A. donax* biotypes which originated from Alicante, Spain, the same region of origin of the agent, were most suitable for *R. donacis* survival (Goolsby *et al.* 2013a). However, survival was significantly lower on the *A. donax* biotype which was genetically distant to *A. donax* populations from Alicante where *R. donacis* was collected (Goolsby *et al.* 2013a), suggesting that biotype compatibility has the potential to influence *R. donacis* survival. Pre-release efficacy testing conducted in the field in the native range of *R. donacis* and in a quarantine laboratory in the USA revealed that *R. donacis* has the potential to have a significant impact on the weed's lateral shoot growth, rhizome weight and photosynthetic ability (Cortés *et al.* 2011a; Moran *et al.* 2011).

Rhizaspidiotus donacis is currently a candidate biological control agent for *A. donax* in South Africa. Collections of *R. donacis* from Alicante, Spain were made available for biological control research on *A. donax* in South Africa, through partnership with the USDA-ARS *A. donax* biological control program. Whilst South African *A. donax* or ‘Spaanseriet’ populations in South Africa are believed to have originated from Spain, this remains unconfirmed. It is known that *A. donax* populations from both Mediterranean Europe, including Spain, and South Africa have a M1 haplotype (Canavan *et al.* in press). However, it is unknown whether South African *A. donax* originated from a region in Spain or elsewhere in the Mediterranean. The genetic proximity of *A. donax* from South Africa to *A. donax* populations from different locations in Mediterranean Europe is also unclear. Therefore, the suitability of *R. donacis* sourced from Alicante, Spain for the biological control of *A. donax* in South Africa is unknown.

The aim of this study was to determine the suitability of *R. donacis* from Alicante, Spain for the biological control of *A. donax* in South Africa. The study’s objectives were to measure the: (1) level of survival of *R. donacis* on South African *A. donax* plants; (2) effect of herbivory by *R. donacis* on plant growth parameters (i.e. rhizome mass, main stem length, leaf production, lateral shoot production and lateral shoot length) of South African *A. donax* plants and (3) effect of herbivory by *R. donacis* on plant growth parameters (i.e., main stem length and leaf production) of South African *A. donax* plants over time.

3.2. METHODS

3.2.1. Plant collection and care

A site located in Pietermaritzburg (S 29°35.254' E 030°22.716') in the province of KwaZulu-Natal was selected for harvesting 30 *A. donax* rhizomes to be used in the trial. Rhizomes were removed from the ground using garden forks and picks and were then transported to the biological control research facility of the Agricultural Research Council – Plant Protection Research Institute (ARC-PPRI) for planting. *Arundo donax* rhizomes of similar weight ($0.371 \pm 0.01\text{kg}$; mean \pm SE), were individually planted in eight-litre plastic tubs, filled two thirds with sand. A small portion of the top of each rhizome was left exposed. Approximately 20 grams of Multicote™ fertiliser was placed on the surface of the sand and the plants were manually watered twice daily, taking care to avoid waterlogging. This was on

the recommendation of J. Goolsby (USDA-ARS), who suggested that the scales may not be very tolerant to waterlogged soils. The potted rhizomes were maintained in a quarantine greenhouse with a constant controlled temperature of 27°C and 60% relative humidity. The growing conditions followed closely those specified by (Goolsby *et al.* 2013a).

3.2.2. Insect collection

Reproductive adult females of *R. donacis* were supplied from native range field collections in Alicante, Spain by the Beneficial Insects Research Unit of the USDA-ARS in February 2016. The methods used to process *R. donacis* scales closely followed those specified by Goolsby *et al.* (2013a). Fine forceps were used to remove the female scales from the *A. donax* rhizomes and isolate them in gelatine capsules (5-10 per capsule), which were then maintained in a growth chamber set at $28 \pm 1^\circ\text{C}$ and a photoperiod of 14:10 (L:D) to facilitate the emergence of crawlers. Screening for parasitism was conducted for all female scales by checking the gelatine capsules daily and removing and destroying those in which parasitoids had emerged.

3.2.3. Survival trials

The methods used in these trials followed closely those specified by Goolsby *et al.* (2013a). To evaluate the survival of *R. donacis*, 10 of the test plants were maintained for up to 60 days after infesting them with crawlers, with 10 maintained for up to 140 days. These constituted the experimental plants, whilst an additional 10 plants were maintained as uninfested control plants. Each experimental plant was infested with approximately 1000 live crawlers, one week after planting. The experimental plants were not irrigated for two days before and after infestation, to provide suitable conditions for crawler settling. To obtain crawlers, gelatine capsules containing isolated adult females were checked daily for crawler emergence. Daily counts of crawlers were conducted, after which the females were transferred to new gelatine capsules and the crawlers were transferred to the experimental plants. This was done by pinning gelatine capsules containing the isolated crawlers onto the rhizomes with developing buds. The capsules were removed once it was confirmed by observation that all the crawlers had moved onto the experimental plants. It took 1-2 days to infest each experimental plant with the desired number of crawlers.

In order to determine the survival of *R. donacis*, destructive sampling was conducted on both subsets of experimental plants on the 60th or 140th day after infestation. These

dissections coincided with the dissection of control plants. This process involved removing and dissecting the rhizomes, leaf collars and the bases of axillary shoots, and carefully inspecting and removing *R. donacis* individuals from them. Gender identifications of the scales were made according to the size and shape of the scale coverings. Scales with round coverings and white caps were classified as females and scales with oval coverings as males (Moran & Goolsby 2010).

3.2.4. Measurements of impact on plant parameters

The impact of *R. donacis* on *A. donax* was monitored by recording selected plant parameters on the experimental plants that were infested for 60 days and the control plants. The 60-day experimental plants were used to assess impact, as the fast-growing nature of *A. donax* often results in plants becoming dry, and often dying, after prolonged periods of being pot-bound. Therefore, assessing impact over a longer time could have added confounding factors to the impact measurements. The wet rhizome mass, main stem length and number of leaves, was measured for each of the experimental and control plants at the initiation and termination of the trial period. The total number of lateral shoots and lateral shoot length was measured for each of the experimental and control plants at the termination of the trial period, since no lateral shoots were present at the initiation of the trials. Relative growth rates (RGRs) ($= \text{final} - \text{initial measurement} / \text{time}$) were determined for wet rhizome mass and main stem length. Furthermore, after the termination of the trials, rhizomes from the experimental and control plants were oven dried at 80°C for 2-3 weeks until constant dry mass was attained.

Additionally, the impact of *R. donacis* on *A. donax* was monitored by measuring the main stem length and number of leaves on the experimental and control plants over time. There were four repeated measurements which took place at two-week intervals. Lateral shoot production and lateral shoot length were excluded from these repeated measurements as lateral shoots were only produced by test plants closer to the termination of the trials. The six-week repeated measurements did not mark the termination of the impact trials but only the final repeated measurements. Dissections of experimental and control plants and final impact measurements were conducted on different days, 2-3 weeks after the final repeated measurements. Weight and length measurements were conducted using a hanging scale and tape measure, respectively.

3.2.5. Statistical analysis

Survival trials

Statistical analysis was conducted using IBM SPSS Statistics 23.0 and Microsoft Excel 2016. For the survival trials, comparisons were made of total survival counts, male counts and female counts between the two sets of experimental test plants (i.e. infested for 60 days and 140 days), to confirm that any differences were not significant. The data sets did not meet the assumptions for normality and equality of variances, so Mann-Whitney U tests were used to compare the means and confirm that survival was consistent between the two trials.

The scale counts for both sets of experimental treatments were then combined and comparisons were made between male counts and female counts using a Mann-Whitney U test as the assumptions of normality and equality of variance were not met. The percentage of crawlers that survived ($= \text{number of scales at the end of the trial} / \text{number of crawlers released at the start of the trial} \times 100$) was calculated for the survival trials and compared to survival recorded during separate host-specificity tests with the host plant (A. Bownes unpublished data). Test plants used in the survival trials were infested with approximately 1000 crawlers for 60 or 140 days, while those in the host-specificity trials were infested with approximately 200 crawlers for 105 days. Percentage survival was compared between the survival trials and the host-specificity trials using a Mann-Whitney U test.

Impact trials

For the impact trials, comparisons of wet rhizome mass, main stem length and leaf production at the initiation and termination of the trial period were made between the experimental (i.e. infested for 60 days) and control (i.e. uninfested) plants. Assumptions of normality and equality of variance were met for all parameters except final wet rhizome mass where the data were not normally distributed. Where the assumptions were met, independent samples t-tests were used, otherwise a Mann-Whitney U test was used.

Since there were no lateral shoots at the initiation of the trials, comparisons between experimental and control plants were made for lateral shoot production and total lateral shoot length, at the termination of trials. Assumptions of normality and equality of variances were met, allowing independent sample t-tests to be used for these comparisons. Comparisons were made of RGRs of wet rhizome mass and main stem length between the experimental and control plants using independent samples t-tests, as the assumptions of normality and equality of variance were met. Additionally, comparisons of dry rhizome mass at the termination of the

trials were made between the experimental and control plants using independent sample t-tests as the assumptions of normality and equality of variance were met.

A 2 x 4 mixed design Analysis of Variance (ANOVA) was used to analyse the effect of time and treatment on plant parameters (i.e. main stem length and leaf production) which were measured repeatedly over time. The number of weeks (i.e. 0 to 6) was used as the within subject's variable, and treatment (i.e. experimental and control) as the between subject's variable. Whilst the assumptions of equality of variances were met, the assumptions of sphericity were violated. Therefore, the corrected Greenhouse-Geisser F-values, which account for lack of sphericity, were reported.

3.3. RESULTS

3.3.1. *Rhizaspidiotus donacis* survival

Counts of crawlers which survived to their subsequent life stages for the 60-day and 140-day dissections were combined as there was no significant difference between these treatments in the mean number of scales that established on the plants ($U = 38.000$, $n = 20$, $P = 0.393$), including male numbers ($U = 43.500$, $n = 20$, $P = 0.631$) and female numbers ($U = 42.500$, $n = 20$, $P = 0.579$). Furthermore, Moran & Goolsby (2010), suggested that the survival of adult females varies little beyond 60 days after crawler settling.

The mean number of males and females recorded per plant were 14.05 ± 3.58 and 20.00 ± 7.96 (mean \pm SE, $n = 20$), respectively and the differences were not significant ($U = 177.0$, $n = 40$, $P = 0.529$). The mean number of adults per plant (i.e. males and females) was thus 34.05 ± 9.81 (mean \pm SE, $n = 20$). Crawler survival from infestation to trial termination was only 3.41 ± 0.98 % (mean \pm SE, $n = 20$) which was very similar to the 3.22 ± 1.20 % survival recorded in the host-specificity trials (mean \pm SE, $n = 9$). There was no significant difference in the percentage crawler survival between the survival trials and the host-specificity trials ($U = 84.500$, $n = 29$, $P = 0.799$) (Fig 3.1.). The percentage crawler survival on the different US *A. donax* genotypes, in the study conducted by Goolsby *et al.* (2013a), was far higher than that obtained in these survival trials and the host-specificity trials (

Appendix III).

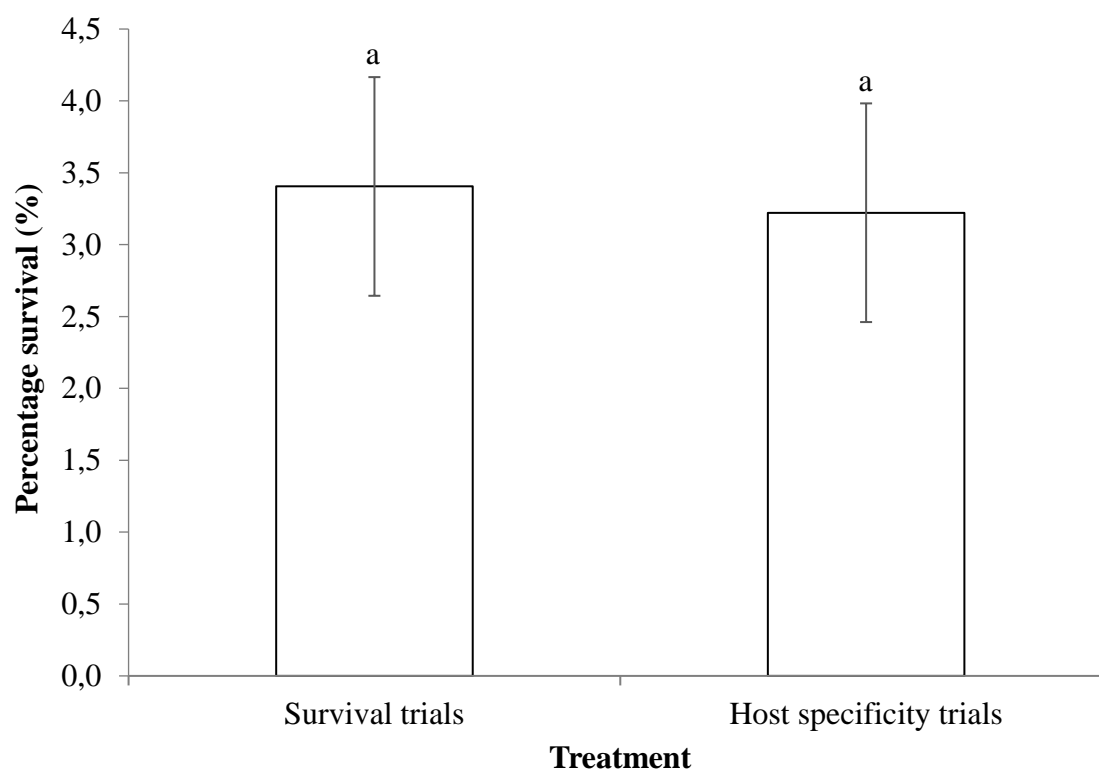


Fig 3.1. Percentage of *Rhizaspidotus donacis* crawlers from Alicante, Spain that survived to adulthood on *Arundo donax* during the survival trials and host-specificity trials. Means (\pm SE) followed by the same letters are not significantly different ($P > 0.05$).

3.3.2. Impact of *Rhizaspidotus donacis* on *Arundo donax*

Both the experimental and the control plants used to evaluate the impact of *R. donacis* herbivory were of similar size at initiation of the trials. There was no significant difference in the initial wet rhizome mass, main stem length and leaf production between the experimental and control plants (Fig 3.2; Table 3.1.). There were no lateral shoots at the initiation of the trials. There was no significant difference in the final wet rhizome mass, main stem length and leaf production between the experimental and control plants (Fig 3.2; Table 3.1.). There was no significant difference in the final lateral shoot production and lateral shoot length between the experimental and control plants at the termination of the trials (Fig 3.3; Table 3.1.).

Table 3.1. Statistical details from comparisons of plant parameters between the experimental and control plants.

Plant parameter	Test statistic	d.f.	n	<i>P</i> -value
Initial wet rhizome mass	$t = 0.440$	7	9	0.673
Initial main stem length	$t = -0.170$	7	9	0.870
Initial number of leaves	$t = -0.717$	7	9	0.497
Final wet rhizome mass	$U = 8.500$	7	9	0.712
Final main stem length	$t = -0.070$	7	9	0.946
Final number of leaves	$t = -0.308$	7	9	0.767
Final number of lateral shoots	$t = 0.318$	7	9	0.760
Final lateral shoot length	$t = -0.020$	7	9	0.984

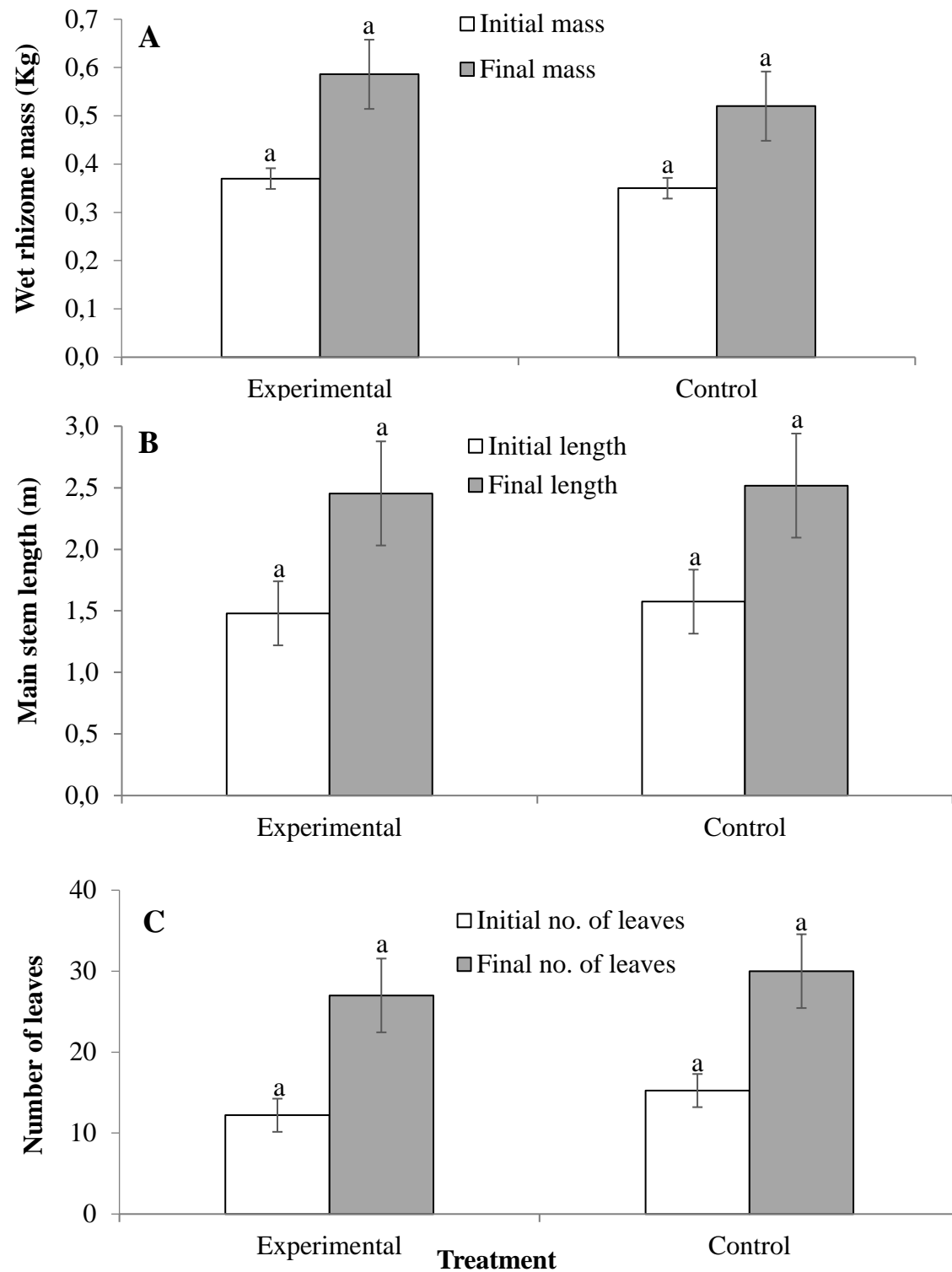


Fig 3.2. Impact of herbivory by *Rhizaspidiotus donacis* from Alicante, Spain on *Arundo donax* growth parameters as determined by (A) wet rhizome mass, (B) main stem length and (C) leaf production. Means (\pm SE) compared between experimental and control treatments; those followed by the same letters are not significantly different ($P > 0.05$).

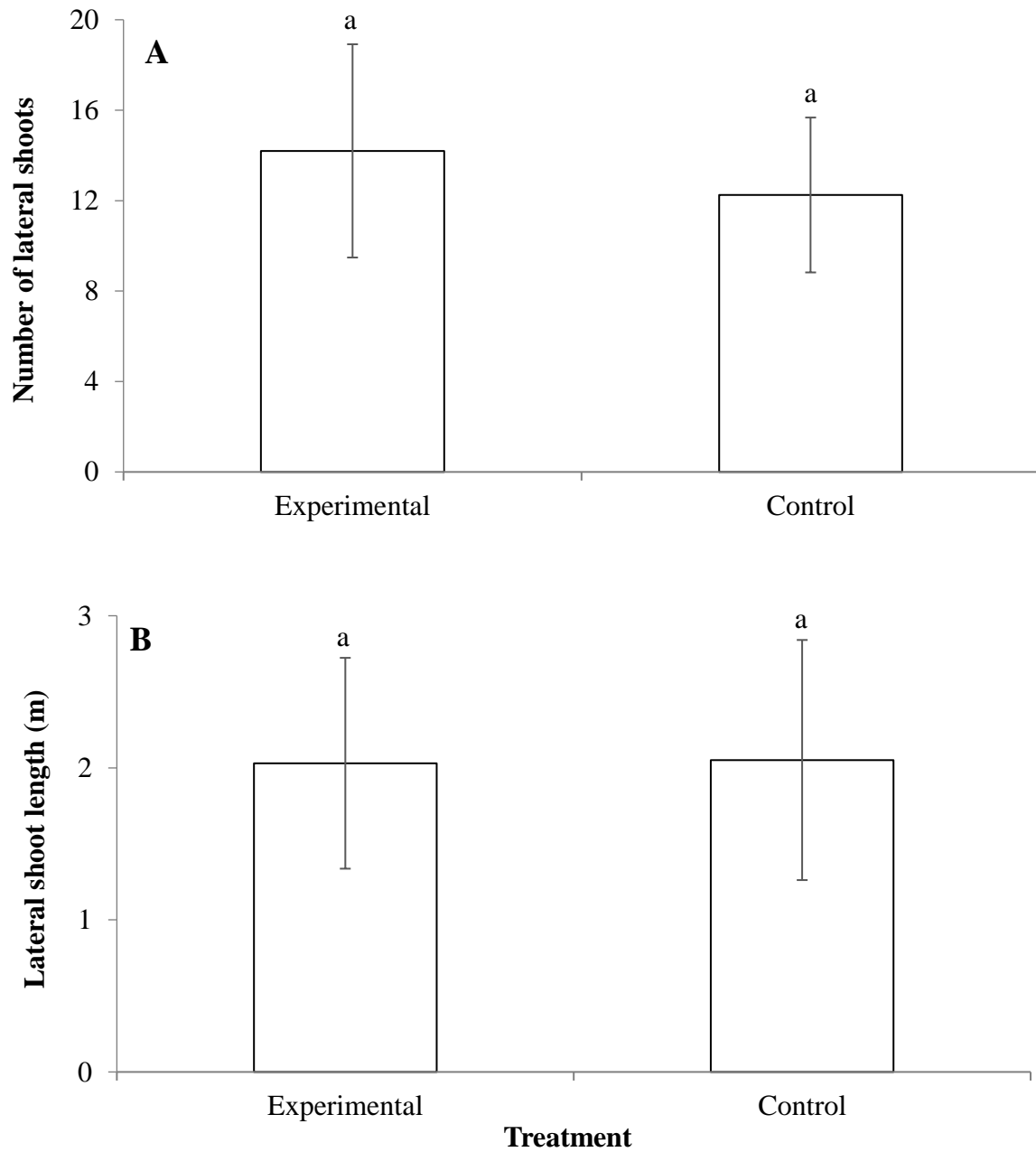


Fig 3.3. Impact of herbivory by *Rhizaspidiotus donacis* from Alicante, Spain on *Arundo donax* growth parameters as determined by (A) final lateral shoot production and (B) final lateral shoot length. Means (\pm SE) followed by the same letters are not significantly different ($P > 0.05$).

Consequently, there was no significant difference in the RGRs of the experimental and control plants (Fig. 3.4.) in terms of their main stem length ($t = 0.120$, $n = 9$, $P = 0.907$) and wet rhizome mass ($t = 0.357$, $n = 9$, $P = 0.731$).

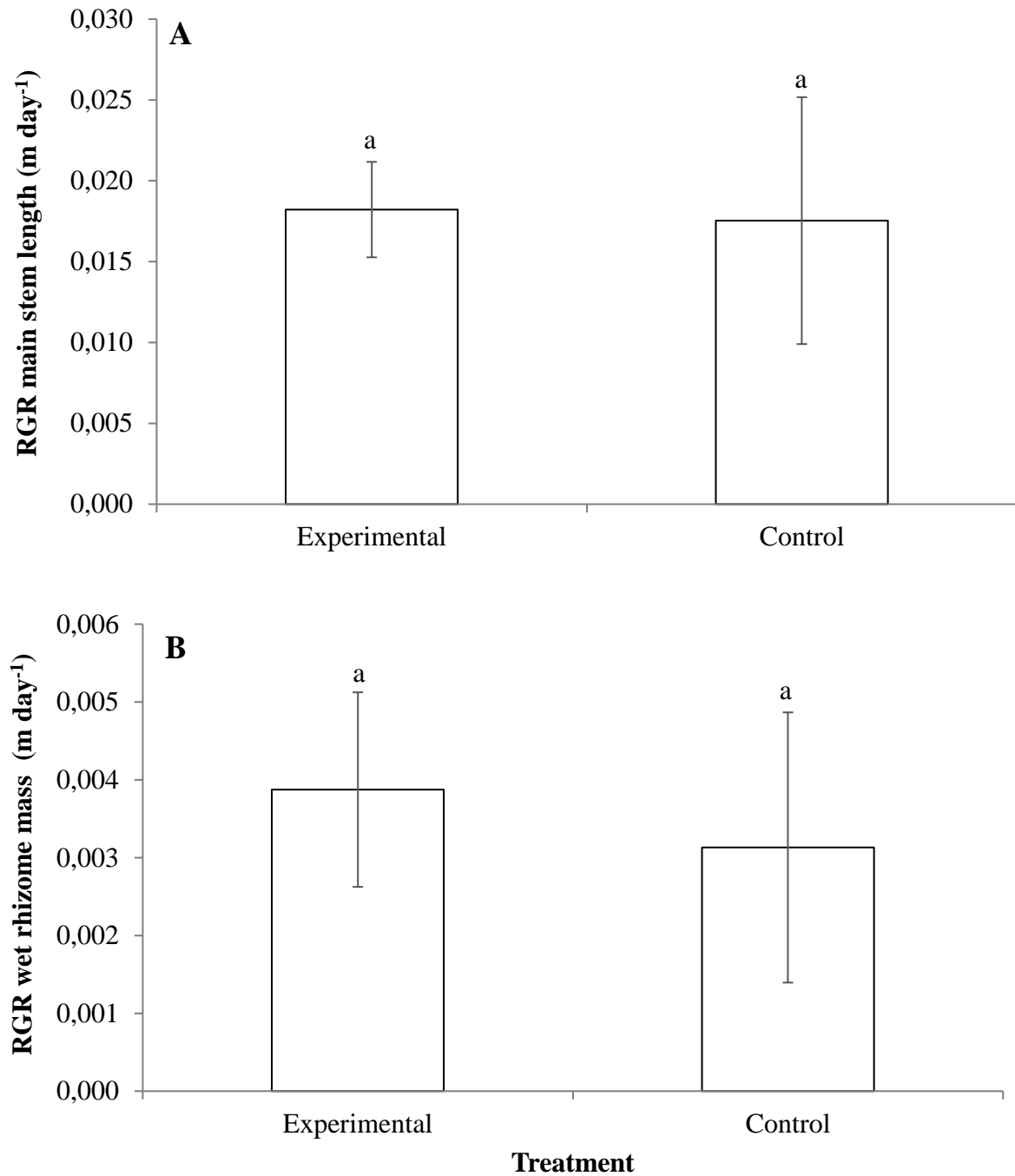


Fig 3.4. Impact of herbivory by *Rhizaspidiotus donacis* from Alicante, Spain on *Arundo donax* as determined by the relative growth rate of (A) main stem length and (B) wet rhizome mass. Means (\pm SE) followed by the same letters are not significantly different ($P > 0.05$).

Since there were no significant differences between the experimental and control treatments in the final wet rhizome mass or the RGR of wet rhizomes mass and since the mean final wet rhizome mass in the experimental treatment was slightly higher than in the control treatment,

dry rhizome masses were compared. Although not significantly different ($t = -1.289$, $n = 9$, $P = 0.238$), the dry rhizome mass was somewhat higher in the control treatment than in the experimental treatment (Fig 3.5.).

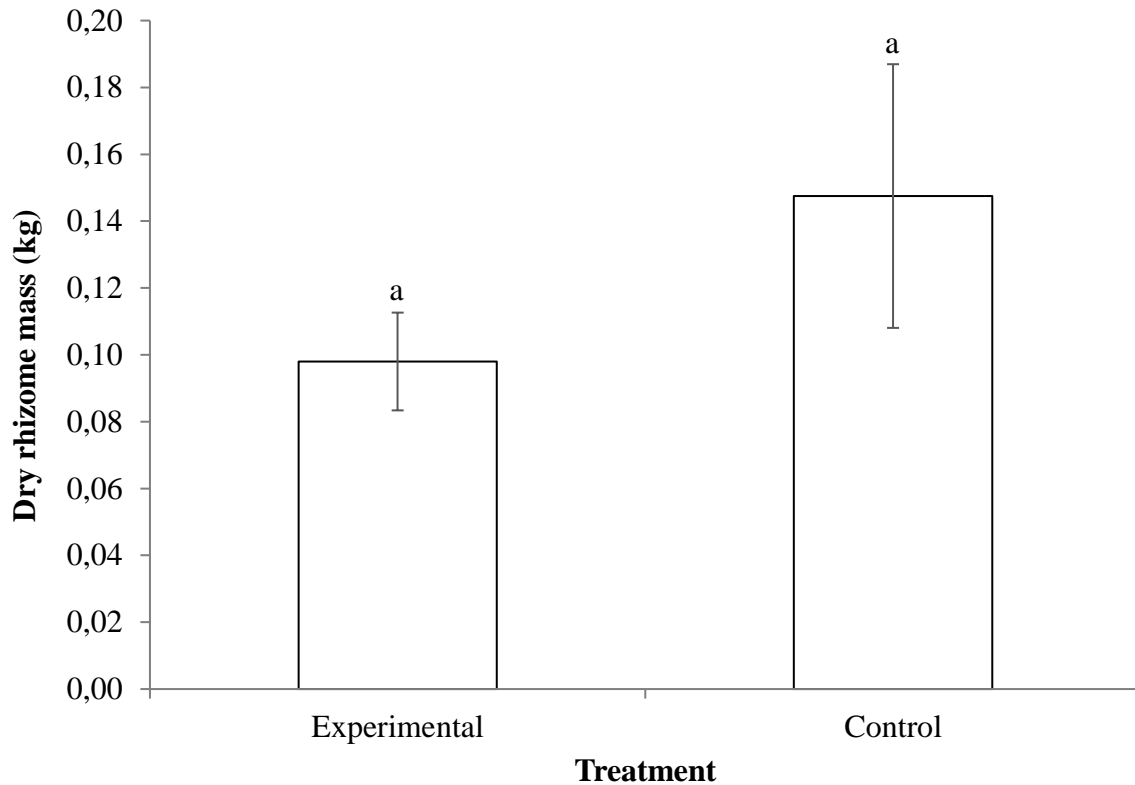


Fig 3.5. Impact of herbivory by *Rhizaspidiotus donacis* from Alicante, Spain on *Arundo donax* as determined by dry rhizome mass. Means (\pm SE) followed by the same letters are not significantly different ($P > 0.05$).

Mixed design ANOVA's revealed a significant main effect of time on main stem length ($F_{3,21}=18.213$, $P < 0.0005$) and on leaf production ($F_{3,21}=19.911$, $P < 0.0005$). It is noteworthy that a growth spike was observed in main stem length of the experimental plants between the 6th week repetitive measure and the final measurement at the termination of the trials, although there is no clear explanation for this occurrence (Fig 3.2; Fig 3.6.). Although not significant ($F_{1,7}=0.514$, $P > 0.05$), the main stem length was greater in the control plants than in the experimental plants over time (Fig 3.6.). Additionally, there was no significant main effect of treatment on leaf production ($F_{1,7}= 0.781$, $P > 0.05$), despite leaf production being slightly higher in the control plants than in the experimental plants over time (Fig 3.6.). It is noteworthy

that the main stem length and number of leaves was slightly higher in the control plants from the first measurement in the series of measurements.

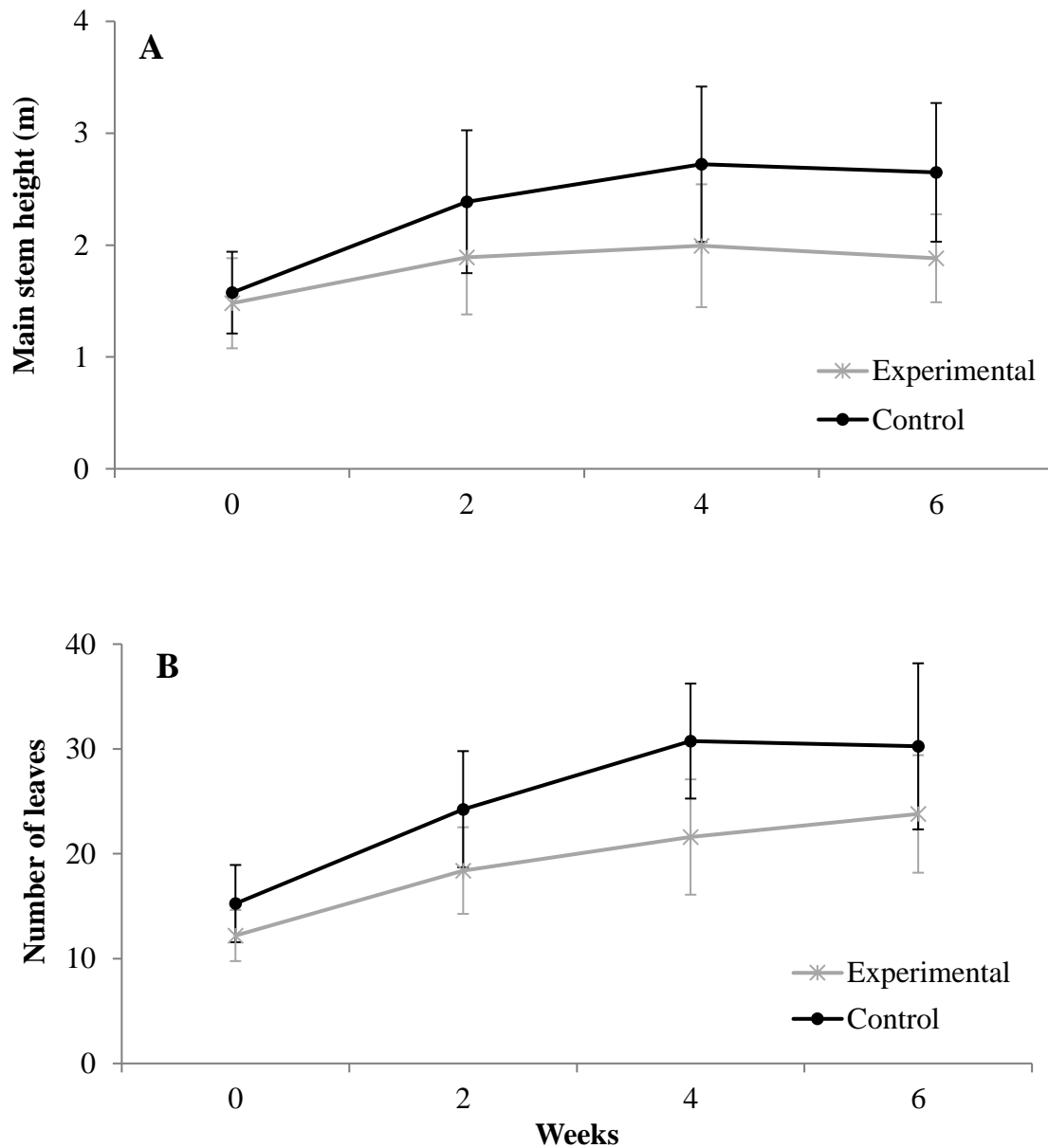


Fig 3.6. Impact of herbivory by *Rhizaspidiotus donacis* from Alicante, Spain on *Arundo donax* over time as determined by the mean (\pm SE) main stem length (A) and leaf production (B) over a six-week period.

3.4. DISCUSSION

Establishment of an agent is one of the first requirements for success in weed biocontrol. Biotype mismatching between the target plant species in its native and invaded range is one possible reason for the failure of agents to successfully establish. This mismatching is the result of candidate biological control agents being collected from host plants that are of a different sub-species or biotype to those found in the invaded range (McFadyen 2003). A well-known example of this is the biological control programme for climbing fern *Lygodium microphyllum* (Cav.) R. Br. (Lygodiaceae), which is invasive in Florida, USA. Populations in the USA are an exact match with populations of the fern in the native range, specifically Cape York, Queensland, Australia. The eriophyd mite, *Floracarus perrepae* Knihinicki & Boczek (Acariformes: Eriophyidae) was evaluated as a natural enemy of *L. microphyllum* (Goolsby *et al.* 2004a). The acceptance of the Florida fern genotype by *F. perrepae* populations from several regions including Cape York, China, Thailand, India and Sri Lanka was evaluated. The Cape York and Thailand *F. perrepae* populations, which came from ferns that were most closely related to the Florida fern genotype, displayed the best performance (Goolsby *et al.* 2004a). Additionally, the failure of the first release of the rubber vine rust, *Maravalia cryptostegiae* (Cummins) Ono, has been attributed to biotype mismatching (Evans & Tomley 1996). Similarly, the failed establishment of Brazilian chrysomelid beetles on *Baccharis halimifolia* L. (Asteraceae) in Australia might have been caused by plant biotype mismatching (McFadyen 2003).

Armoured scale insects tend to have close and well characterised relationships with their host plants and due to their sedentary nature, and can become locally adapted to individual host genotypes (Morse & Normark 2006). *Pseudoaulacaspis pentagona* (Targioni-Tozzetti) (Homoptera: Coccoidea, Diaspididae) is an armoured scale insect that was evaluated for local adaptation to *Morus alba* L. (mulberry) trees in Maryland, USA. The *P. pentagona* survival on various trees suggested that populations were better adapted to surviving on neighbouring *M. alba* trees than on geographically distant *M. alba* trees (Hanks & Denno 1994). Hanks & Denno (1994) suggested that limited mobility and constrained dispersal ability of scale insects may inhibit gene flow across geographical areas and is therefore likely to contribute to the development of local adaptation. Additionally, it was suggested that such local adaptation has the potential to fine-tune herbivore populations to survive on individual host phenotypes

(Hanks & Denno 1994). Therefore, it is likely that the performance of *R. donacis* may be influenced by fine-scale genotypic differences in *A. donax* host populations.

In this study, it was evident that *R. donacis* from Alicante, Spain displayed low levels of establishment and survival on the *A. donax* biotype in South Africa, suggesting a possible mismatch with the *A. donax* biotype from Alicante, Spain. Goolsby *et al.* (2013a) reported significantly higher establishment and survival of *R. donacis* from Alicante, Spain on the *A. donax* biotype from Austin, Texas which was phylogenetically close to *A. donax* from Alicante Spain, as compared to the phylogenetically distant Balmorhea, Texas biotype. The Austin *A. donax* biotype is believed to originate from coastal Spain, near Alicante, whilst the Balmorhea *A. donax* biotype originates from elsewhere. More noteworthy was the significantly greater establishment levels of *R. donacis* from Alicante, Spain on the *A. donax* biotype from Austin, Texas than on the *A. donax* biotype from Laredo, Texas (Goolsby *et al.* 2013a). These findings are especially interesting as both these biotypes are closely related and are both of Spanish origin, yet significantly different settling levels were observed. Survival and establishment can thus be significantly influenced by both fine-scale and large-scale genotype differences. Furthermore, it supports the notion that the South African *A. donax* biotype and the *A. donax* biotype from Alicante, Spain may be mismatched. The South African plants may have originated from elsewhere in Spain or Mediterranean Europe and it is likely that they are not phylogenetically close to *A. donax* from Alicante, Spain. Based on the findings in the USA, we would expect *R. donacis* from the region of origin of South African *A. donax*, with close phylogenetic proximity, to display higher establishment and survival and thus be the most suitable biotype for biological control. This highlights the need to determine, as closely as possible, the area of origin of South African *A. donax*.

However, there are other possible factors that could have caused the low establishment levels of *R. donacis* in these and the host-specificity trials. Crawlers are responsible for the dispersal and survival of armoured scale species. If crawlers do not survive long enough to settle on their host plants, they cannot develop into adults. Several factors influence crawler behaviour including the availability of suitable settling sites, plant genotype suitability (Goolsby *et al.* 2013a) and environmental conditions such as the nature of the settling substrates, temperature, light, wind and humidity (Rosen 1990). Adult scales were mostly found on hairy leaf collars and on rough concealed segments of the rhizomes of the *A. donax* test plants. This is consistent with the notion that crawlers have a preference for rough settling surfaces (Rosen 1990). It is thus possible that the low establishment observed during this study

could have been influenced by insufficient suitable and rough settling sites being available. In addition, the environmental conditions under which the plants were maintained, including soil moisture levels, could have affected the settlement or development of crawlers. Additionally, these trials and the host-specificity trials marked the first work done with *R. donacis* in South Africa, and although guidelines were available from the biological control program in the USA, no hands-on experience was available. Therefore, the survival and establishment of *R. donacis* scales could have been influenced by the inability to replicate the most suitable conditions required for development and breeding of *R. donacis* in quarantine. However, it is noteworthy that crawler survival levels were very similar, and not significantly different, between the survival trials and the host-specificity trials, which had differing planting methods and watering regimes. This could possibly support the notion of a biotype mismatch and suggests a high likelihood that a genetically unsuitable South African *A. donax* biotype is responsible for the low establishment levels of *R. donacis* from Alicante, Spain.

Pre-release experimental studies are useful in providing an indication of an agent's potential to affect growth parameters of a target weed (Morin *et al.* 2009) and agent per-capita damage (Sheppard *et al.* 2003). Despite such studies providing different measurements compared to field impact studies, they do contribute to understanding population level impacts (Sheppard *et al.* 2003). In this glasshouse study, *R. donacis* from Alicante, Spain failed to demonstrate significant reductions in any of the measured plant growth parameters or relative growth rates of South African *A. donax* plants. It was noteworthy that dry rhizome mass was lower in experimental plants than in control plants and that main stem length and leaf production was lower in experimental plants than in control plants over time. Whilst these findings may infer that *R. donacis* herbivory could have had some impact on rhizome biomass and both main stem length and leaf production over time, the results should be interpreted with caution as they lacked statistical significance. Goolsby *et al.* (2009b) assessed the combined impact of the wasp, *T. romana* and *R. donacis* on *A. donax* under quarantine conditions during a 12-week period. Whilst *T. romana* alone caused a significant impact on stem and leaf growth, the combined impact of both agents on growth was only slightly, but not significantly, higher. This suggested that *T. romana* has the potential to have a substantial impact within a short period of time, whilst *R. donacis* does not, possibly due to its longer life cycle. Consequently, longer time periods may be required for *R. donacis* to have a measurable impact (Goolsby *et al.* 2009b).

In contrast to these glasshouse studies, field studies in the native range did show significant reductions in plant growth parameters of *A. donax* due to *R. donacis* (Cortés *et al.* 2011a; Cortés *et al.* 2011b). The size of rhizomes from sites with and without *R. donacis* in Spain and France was compared and showed that *A. donax* rhizomes from sites with *R. donacis* weighed 50% less than those from sites without *R. donacis* (Cortés *et al.* 2011b). *Arundo donax* shoot growth was also evaluated in a manipulated field experiment in the native range, by measuring the length of *R. donacis*-infested shoots and insecticide-treated shoots. Significant reductions in growth of *R. donacis*-infested shoots suggested that *R. donacis* has the ability to cause significant damage to *A. donax* shoot growth (Cortés *et al.* 2011a). *Rhizaspidiotus donacis* populations in the native range, where these studies were conducted, were established over an extended period (i.e. multiple years) and had completed several generations on the *A. donax* populations. It is thus possible that the significant impact recorded during these studies may be due to the duration of the *R. donacis*-*A. donax* relationship in the native range. This supports the notion that *R. donacis* populations may need to develop on *A. donax* populations for extended periods, and undergo several generations before significant impact is demonstrated (Goolsby *et al.* 2009b). Although this will take longer, if it results in significant reductions in rhizome size, *R. donacis* could prove highly effective, as the primary mode of *A. donax* spread is through vegetative growth of rhizomes. However, if the low survival of *R. donacis* from Alicante, Spain on South African *A. donax* is due to a biotype mismatch, it could preclude population increases over time, thus making it ineffective. If this is the case, a better matched *R. donacis* biotype that displays higher settling rates and survival should prove more effective.

The crawler infestation level (i.e. 57 125 crawlers per week per stem over 12 weeks) in the study by Goolsby *et al.* (2009b), was substantially higher than the infestation level in this study (i.e. 1000 crawlers per plant). However, neither of these laboratory studies demonstrated a significant impact of *R. donacis* on *A. donax*. Goolsby *et al.* (2009b) suggested that the low impact of *R. donacis* may have been due to crawler numbers being insufficient to cause significant damage. This suggests that substantially higher numbers than used in both of these studies may be required to cause significant impacts on *A. donax*. The low establishment levels of *R. donacis* from Alicante, Spain on South African *A. donax* will make it substantially more difficult for populations to establish and reach population densities that will have a significant impact on *A. donax*. In addition, this poses logistical challenges associated with collecting and

rearing high numbers of a possibly less suitable biotype of *R. donacis*, and would not be cost-effective.

In conclusion, this study has provided evidence that *R. donacis* from Alicante, Spain lacks the potential to sufficiently establish on South African *A. donax*. One probable reason is the lack of biotype suitability of the South African *A. donax* biotype to sufficiently support *R. donacis* collected from the *A. donax* biotype in Alicante, Spain, as *R. donacis* may be locally adapted to *A. donax* in this region. Other regions in Spain, or elsewhere in the Mediterranean, may host *A. donax* populations that are genetically closer to *A. donax* populations in South Africa. *Rhizaspidiotus donacis* collections sourced from *A. donax* populations with close phylogenetic proximity to South African *A. donax*, or from the population of origin of *A. donax* in South Africa, are likely to display the highest levels of suitability. Additionally, survival of *R. donacis* could have been compromised by the required culturing conditions not being fully achieved. However, there is the possibility that both biotype mismatching and unsuitable culturing conditions could have been responsible for the observed *R. donacis* survival levels. As a result, the study failed to demonstrate significant impact of *R. donacis* on the plant growth parameters assessed. However, based on studies in the native range, a suitable biotype of *R. donacis* could have the potential to have a substantial impact on the growth of *A. donax* when populations are able to reach high densities and complete several generations. However, such impacts in an area of introduction are only likely to be realized with the use of a suitably matched *R. donacis* biotype and sufficient establishment and survival levels. Genetic studies have been useful in determining that *A. donax* in South Africa is of the M1 haplotype (Canavan *et al.* in press). However, the precise origin of the *A. donax* genotype in South Africa and its genetic proximity to Spanish and Mediterranean *A. donax* populations have yet to be determined. It is recommended that future endeavours focus on determining the genetic origin of South African *A. donax* populations and their phylogenetic proximity to *A. donax* populations in regions in Spain and elsewhere in Mediterranean Europe, from which *R. donacis* stocks can be sourced. Additionally, culturing conditions should be evaluated to determine those which promote optimum *R. donacis* settlement and survival.

CHAPTER 4: GENERAL DISCUSSION AND CONCLUSIONS

The focus of this study was to assess the suitability of *R. donacis* for the biological control of *A. donax* in South Africa in terms of its climate suitability and performance in terms of its survival and impact on the target weed. Climate suitability was assessed through climate modelling of *A. donax* and *R. donacis* using the CLIMEX software programme, while survival and impact of *R. donacis* from Alicante, Spain was evaluated through experimental testing under quarantine conditions. This chapter summarises the major findings, limitations and conclusions of these evaluations and discusses the implications and recommendations thereof.

4.1. CLIMATE SUITABILITY

4.1.1. Fundamental distribution of *Arundo donax*

It is important to know the potential geographical distribution of an invasive weed and the land uses and climate types in which the weed is problematic (Nordblom 2003). The results presented in chapter 2 indicate that *A. donax* is likely to have realised the majority of its potential distribution in the Mediterranean, humid sub-tropical, marine west coastal and semi-arid climatic regions of South Africa. Furthermore, although arid regions are likely to be climatically unsuitable for *A. donax*, the weed has the ability to thrive in areas with additional water inputs, such as in riparian areas or in croplands. Therefore, it is apparent that although soil moisture is an important limiting factor for *A. donax*'s spread into drier areas, *A. donax* has the potential to spread into riparian habitats in arid regions. Furthermore, *A. donax* is adapted to several distinctive climate types including Mediterranean, temperate, tropical, subtropical and arid regions (Spencer *et al.* 2010; Cortés *et al.* 2011a; Cortés & Marcos-García 2012), which has been confirmed by the findings of this study. The adaptability of *A. donax* to a wide range of climate types is a common characteristic of invasive plants (Clewley *et al.* 2012).

Having a greater understanding of the actual distribution of a weed in relation to its potential distribution allows a better understanding of the current and future threat of the weed. The findings of this study highlighted the seriousness of existing *A. donax* invasions, in terms of their potential to spread into riparian areas or to become denser where already present. However, low soil moisture or drought have the potential to limit *A. donax* spread. It is apparent

that current management methods have been ineffective in curbing the spread of *A. donax* in South Africa. Therefore, biological control is likely to be the best option as it is evident that more effective and active control is required. Furthermore, biological control may be the only possible solution for this widespread IAP that has the potential for further spread. The information on the fundamental distribution of *A. donax* in South Africa can contribute to the *A. donax* biological control program, by allowing comparisons to be made with the predicted distributions of *R. donacis* or other potential agents which may be considered in the future. These comparisons can help to identify the extent to which potential agents are likely to be able to control *A. donax* in specific areas (Kriticos 2003).

Based on these inferences, it is recommended that the control of *A. donax*, particularly biological control, be pursued more actively and with greater urgency in South Africa. Furthermore, current *A. donax* infestations should be monitored closely in order to prevent, where possible, increases from low or moderate levels of invasion to high levels of invasion. Particular attention should be given to monitoring riparian habitats in arid regions which are at risk of being invaded by *A. donax*. South Africa is a water scarce country which is threatened by frequent droughts and could be worsened by *A. donax* infestations which pose a serious threat to the scarce water resources. Furthermore, drought periods are likely to render *A. donax* more vulnerable and may be the best time to exert control. However, this will be dependent on the control methods deployed and in the case of biological control, the agent's phenology. If *R. donacis* is able to persist in drought conditions, it could be effective during such periods.

4.1.2. Climate suitability of South Africa for *Rhizaspidiotus donacis*

Establishment and proliferation of an agent in the country of introduction are fundamental to biological control success (McFadyen 2003). The potential distribution of the agent in comparison to the potential distribution of the target is fundamental to agent impact (Kriticos 2003). This aspect is important as it determines what proportion of the weed's distribution can be controlled by the prospective agent (Kriticos 2003). Comparisons of the potential range of the biological control agent *A. hygrophila* and its target weed, *A. philoxeroides*, were conducted in Australia. The model helped to identify regions where the agent was predicted to have an effect on the target weed, and regions where other control methods were required (Julien *et al.* 1995). As demonstrated in chapter 2, the majority of the distribution range of *A. donax* in South Africa is likely to be climatically suitable for *R. donacis*. Furthermore, *R. donacis* is likely to persist in arid areas which were predicted to be unsuitable,

since *A. donax* is only likely to persist in these areas with continuous exposure to additional water inputs.

The abundance of weed biocontrol agents is influenced by their interaction with climate and the biotic environment. Therefore, predictions of agent abundance can benefit from climate modelling and knowledge of agent ecology (Sheppard *et al.* 2003). Approximately 40% of released agents fail to establish due to climate incompatibility with the region of introduction, which results in wasted resources and time (Byrne *et al.* 2004). The findings of this study are useful as they provide a preliminary indication of the suitability of *R. donacis* for biological control in South Africa based on climatic compatibility. The availability of this information early in the programme helps to avoid substantial wastage of resources that result from testing and releasing an agent which fails to establish due to climate incompatibility. The predicted establishment of *R. donacis* over most of the range of *A. donax* demonstrates the suitability of the agent in terms of climatic adaptability. Furthermore, in the event that *R. donacis* is approved for release against *A. donax* in South Africa, release strategies can be designed around these findings. It is recommended that priority release sites are selected by considering the areas that have the worst infestations in relation to those that are likely to be climatically most suitable. Additionally, initial releases should also be focused in areas that are easily accessible for cost-effective monitoring of agent establishment. Based on these considerations, it is recommended that initial releases be focused along the coast of KwaZulu-Natal Province. These regions have high levels of *A. donax* invasion, are predicted to be climatically suitable for the agent and can be easily accessed by biological control practitioners in the province. Model predictions of the climatic suitability of different areas for agents can be used to determine the best sites for release, and to allow redistributions to be more efficient (Kriticos 2003). From these extrapolations, *R. donacis* can be recommended as a suitable agent on the basis of climate.

4.2.3. Limitations of climate models

There is a strong relationship between the data used for modelling and the outputs of the models. Predicted distributions are closely related to the type of distribution data used and the proportion of native and introduced range distribution points used is likely to have a major influence on the suitability predictions. A greater proportion of native distribution data will be more biased towards native range climates and vice versa (Shabani & Kumar 2015). It is suggested that the maximum number of distribution points from both native and introduced ranges should be used when conducting such species distribution modelling. The lack of

sufficient points can lead to distorted potential distribution predictions; therefore, it is best to have complete datasets when conducting such modelling (Shabani & Kumar 2015). For climate modelling of *A. donax* in South Africa, comprehensive distribution data from the native and introduced ranges were used. However, in the case of *R. donacis*, the number of introduced range distribution points was limited as the agent has only recently established in the USA. Therefore, the climate model for *R. donacis* could be somewhat limited in this regard. However, Thuiller (2003) reviewed several modelling methods and noted that no particular method was consistently more accurate than another, even when using different datasets, scales and resolutions. He suggested that the factors contributing to model accuracy are instead species specific as the most accurate models vary from one species to another.

It is important to note that species distributions are not determined by climate alone, but by abiotic and biotic factors as well. The population size of an agent is affected by climate, parasitism and predation (McFadyen 2003). Although predictions based on climate alone can be useful, more realistic results are achieved if other non-climatic parameters, such as land use, slope and soil properties are included (Shabani & Kumar 2015). Therefore, some areas which may have been predicted to be suitable for *A. donax* or *R. donacis*, may be unsuitable due to site-specific abiotic and biotic conditions. Land use can have an impact on the presence of *R. donacis* and *A. donax*. For example, in the native range *A. donax* is abundantly present downstream from commercial vegetable greenhouses, while *R. donacis* is not present, possibly due to the effects of residual insecticides and herbicides (Goolsby *et al.* 2013b). Additionally, disturbance has a significant effect on *R. donacis* in the native range. In the absence of disturbance, *R. donacis* is able to establish in high densities on rhizomes below ground and on above ground lateral shoots, and cause substantial damage. However, if plants are cut back by mowing, a substantial portion of *R. donacis* scales are removed from the population and repeated disturbances could result in local extirpation (Goolsby *et al.* 2013b).

4.3. RHIZASPIDIOTUS DONACIS SURVIVAL AND PERFORMANCE

In chapter 3 it was demonstrated that *R. donacis* from Alicante, Spain had limited performance in terms of its survival on the South African *A. donax*. It was inferred that the poor performance could either be attributed to biotype mismatching or unsuitable culturing

conditions or a combination of these two factors. The major implications of both these possibilities and the recommendations based on them are summarised below.

4.3.1. Biotype mismatching

Weed biological control effectiveness is enhanced by matching agent biotypes with certain strains of their target weeds (Hoffmann 2004). If the poor performance of *R. donacis* is indeed attributed to host biotype incompatibility, *R. donacis* stocks from Alicante, Spain are likely to be ineffective. Furthermore, it is probable that *R. donacis* stocks collected from genetically close *A. donax* populations, or from the population of origin of the South African biotype, are likely to be more compatible. It is a great waste of resources if an agent fails to establish after substantial testing (McFadyen 2003). Ensuring that the most genetically suitable *R. donacis* stocks are used or that the most suitable culturing conditions and requirements are replicated in quarantine testing can prevent resources being wasted from testing and releasing an agent which has limited establishment potential. The best biological control agents are believed to be those which are sourced from native plants in the centre of origin of the target plant species (Evans & Ellison 2004), except in cases of a specific biotype. Furthermore, it is suggested that candidate agents should be sourced from plant species with the closest genetic proximity to the target plant species. This is based on the premise that those agents are likely to be better adapted to the target weed (Day & Urban 2004).

Studying and reconstructing the routes of invasion uncovers vital knowledge on invasion processes, centres of origin and the genetic composition of invasive populations (Estoup & Guillemaud 2010). The lack of such a study for *A. donax* populations in South Africa at the inception of the programme has been a limiting factor in this regard. Whilst it is known that South African populations conform to the M1 haplotype (Canavan *et al.* in press), their precise origin and genetic distance to Spanish and other Mediterranean populations, where *R. donacis* is native, is unknown. It is recommended that a study be conducted to determine the origin of *A. donax* populations in South Africa and their genetic distance to Mediterranean *A. donax* populations that host *R. donacis*. Furthermore, determining the genetic distance to *A. donax* populations in the USA can determine if the same *R. donacis* stocks from eastern Spain, which have been suitable for the USA programme, can be suitable use in for South Africa. Another limitation is the limited number of regions from which *R. donacis* consignments can be obtained through legal contractual agreements, as the programme is still in its early stages,

and consignments which have been received thus far, have been through partnership with the USDA-ARS program.

4.3.2. Culturing conditions

The conditions under which the experimental trials were conducted could have had an impact on the survival of *R. donacis*. If this is the case, the conditions under which *R. donacis* individuals are reared should be adapted to improve survival rates and determine whether *R. donacis* could be an effective agent.

The *R. donacis* biological control programme in South Africa is newly established and the work presented in this study was conducted using the first viable consignment of the candidate agent received in the country. This brought with it limitations which had the potential to affect the performance of the agent. Firstly, the techniques used for the processing, handling and rearing of *R. donacis* in South Africa were based on guidelines and advice from Dr John Goolsby, who is responsible for the USDA-ARS *R. donacis* biocontrol programme. Consequently, there was no previous hands-on experience in working with the agent, and minor differences in rearing conditions could have influenced its survival. However, it is important to note that the guidelines were carefully followed and the study was designed to closely adopt the methods of Goolsby *et al.* (2013a). Secondly, the long life cycle of the agent and the difficulty in obtaining a viable consignment early enough, placed time constraints on the study. Whilst it would have been ideal to study the settling behaviour and development of the candidate agents under varying laboratory conditions, prior to evaluating the variables that formed the basis of this study, time did not allow this.

A study needs to be conducted to evaluate *R. donacis* settlement under differing planting conditions and watering regimes. Soil moisture can influence the presence of *R. donacis* as certain areas may be too wet for *R. donacis* persistence (Goolsby *et al.* 2013b). *Rhizaspidiotus donacis* is present in Syracuse, Sicily where approximately 500 mm of rainfall is received annually and soils are drier, but is not present in nearby mainland Italy where temperatures are warm enough for *R. donacis*, but rainfall is slightly higher. Similarly, in south-eastern France, *R. donacis* is only present in the microclimate of Perpignan where it is protected in the rain shadow of the Pyrenees Mountains. Furthermore, this region has some of the highest *R. donacis* densities, suggesting that lower rainfall and soil moisture are conducive to *R. donacis* persistence (Goolsby *et al.* 2013b). Therefore, it is recommended that particular attention be paid to evaluating the performance of *R. donacis* under different levels of

decreased soil moisture and various watering methods. The findings of this study will help to determine if the low survival rates were caused by culturing conditions in the laboratory or by biotype mismatching. If the performance of *R. donacis* does not improve, it will be indicative of a biotype mismatch. Alternatively, if performance improves, it will provide important information to improve the rearing of the candidate agent.

4.4. RHIZASPIDIOTUS DONACIS IMPACT ON ARUNDO DONAX

In chapter 3 it was noted that the lack of demonstrated impact could have been because such impact would require a longer period of time with the completion of several *R. donacis* generations. The significant impacts recorded on various plant parameters in the native range where *R. donacis* has long been established, maintains high densities and completes several annual generations, supports this inference. Impact studies conducted in the field yield the most realistic impact data (Sheppard *et al.* 2003). Therefore, it is likely that if *R. donacis* is released in South Africa, maintains substantial densities and completes several generations, significant impacts would also be observed. Despite the wait, the benefits are likely to be immense as *A. donax* disperses primarily through vegetative spreading of rhizomes, and *R. donacis* has the potential to cause substantial reductions in *A. donax* rhizome weight (Cortés *et al.* 2011b). Therefore, the biological control of *A. donax* in South Africa by *R. donacis* could reduce and limit the spread of the highly invasive *A. donax*. Much attention is also given to identifying critical plant parts which can be targeted to reduce a target weed's population (McFadyen 2003). An agent is considered to be effective when it causes high levels of damage over a short period of time or when it causes relatively large changes in host plant dynamics with relatively low levels of damage (Sheppard 2003). Since *R. donacis* has the potential to cause large decreases in rhizome biomass, and hence affect the host plant's dynamics, it is likely to be an effective agent.

The uncertainty in this regard is the issue of biotype matching. If, as suggested previously, *R. donacis* from Alicante, Spain is incompatible with South African *A. donax*, then establishment, performance and impact on the plant will be compromised, and release would not be advised. If this is the case, *R. donacis* strains which have higher compatibility with *A. donax* populations in South Africa should instead be released as they would be likely to have significant impacts and be effective in control. However, if *R. donacis* from Alicante, Spain is found to be genetically suitable, then culturing methods should be improved to ensure better

survival levels, like those observed in quarantine studies in the USA prior to release (Moran & Goolsby 2010; Moran *et al.* 2011). Furthermore, *R. donacis* has been released in the USA despite the lack of significant impact under quarantine conditions (Moran *et al.* 2011), and has since contributed to the biological control of *A. donax*, alongside *T. romana*. Recent reports have indicated that higher densities of both agents lead to greater reductions of *A. donax* populations (Avant 2016). Populations of *T. romana* are already present on most *A. donax* populations in South Africa as a result of inadvertent introduction (A. Bowes pers. comm. 2016), suggesting that a suitable strain of *R. donacis* could contribute to the impact already provided by *T. romana*.

One limitation encountered was the fast-growing nature of *A. donax* plants, which often begin to dry out when they are pot-bound for extended periods of time, as adventitious roots become expansive. This could add confounding factors to the impact measurements, so that measuring impact over a longer period of time could not be sustained under quarantine conditions. Since the long-term impacts of *R. donacis* cannot be easily evaluated under quarantine conditions, it is recommended that these be evaluated over shorter periods of time using different densities of a more compatible strain. This might reveal finer-scale differences in impact measurements. In addition, the impact of additional plant parameters such as photosynthetic rates could be evaluated. However, settlement studies and molecular studies of *A. donax* origin should be prioritised above the additional recommended impact studies. These recommended impact studies, together with previous impact studies conducted in the weed's native range and in the USA, could contribute to a greater understanding of the impacts of *R. donacis* over time and under different densities.

Whilst the lack of demonstrated impact under quarantine conditions, such as found in this study, is not a desired result for any prospective agent, it does contribute to the growing knowledge on *R. donacis*. Most importantly, the findings support the notion of possible host incompatibility and the need for longer time periods and higher agent densities to yield significant results.

4.5. CONCLUSION

The focus of this study was to assess the climatic suitability of South Africa for the candidate biological control agent, *R. donacis*, and to assess the survival and impact of *R.*

donacis from Alicante, Spain, on *A. donax* from South Africa. This has been successfully achieved through climate modelling using CLIMEX and comprehensive experimental evaluations conducted under quarantine conditions. Strong evidence has been provided which suggests that the South African climate is likely to support the establishment of *R. donacis* over the range of *A. donax* in South Africa. Despite the predicted suitability in terms of climate, there has been a lack of suitability demonstrated in terms of the survival and impact of *R. donacis* from Alicante, Spain on South African *A. donax*. It has been suggested that the poor performance of *R. donacis* sourced from Spanish *A. donax* is could be due to the lack of phylogenetic relatedness between *A. donax* from Alicante, Spain and South Africa, or unsuitable culturing conditions or a combination of these factors. It has been recommended that further in-depth molecular studies be pursued to determine the exact origin of South African *A. donax* and its genetic proximity to Mediterranean *A. donax* populations. Furthermore, an evaluation of *R. donacis* crawler settlement levels under various planting methods and watering regimes will be pursued in an effort to support the inferences made in this study. Whilst there is potential for the biological control programme in terms of climate suitability, this is only likely to be fully realised when a genetically close and better performing strain of *R. donacis* is found or when higher establishment levels are obtained under quarantine conditions. Further research is thus required to clarify these aspects.

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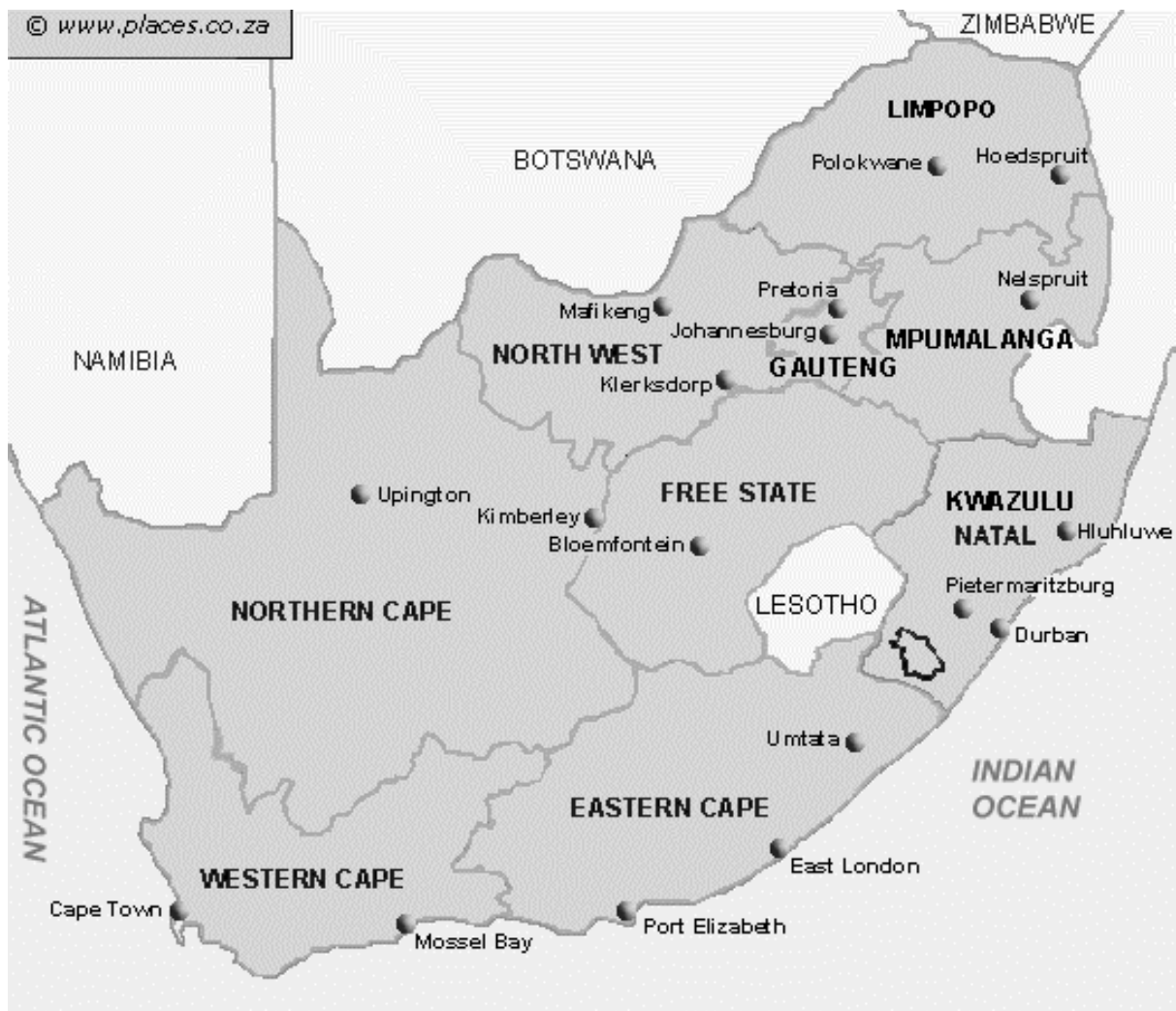
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APPENDICES

Appendix I. CLIMEX parameters for *A. donax* based on native and introduced range distributions (Barney & DiTomaso 2011).

Parameters	Description	Values
<i>Moisture Index</i>		
SM0	Limiting low moisture index	0.1
SM1	Lower optimal moisture index	0.2
SM2	Upper optimal moisture index	2
SM3	Limiting high moisture index	10
<i>Temperature Index</i>		
DV0	Limiting low temperature (°C)	10
DV1	Lower optimal temperature (°C)	20
DV2	Upper optimal temperature (°C)	35
DV3	Limiting high temperature (°C)	40
<i>Cold Stress</i>		
TTCS	Cold stress temperature threshold (°C)	0
THCS	Cold stress temperature rate	-0.0005
<i>Heat Stress</i>		
TTHS	Heat stress temperature threshold (°C)	40
THHS	Heat stress temperature rate	0.002
<i>Dry Stress</i>		
SMDS	Dry stress threshold	0.01
HDS	Dry stress rate	-0.005
<i>Hot-Wet Stress</i>		
TTHW	Hot-Wet temperature threshold (°C)	35
MTHW	Hot-Wet moisture threshold (°C)	1
PHW	Hot-Wet stress rate	0.075

Appendix II. Provinces and key localities in South Africa (Source: www.places.co.za).



Appendix III. Comparison of the percentage survival of *R. donacis* crawlers on different US *A. donax* genotypes and on the *A. donax* biotype from South Africa in the survival trials and preliminary host-specificity trials.

USA Study			Survival trials	Host-specificity trials (Preliminary data)
Austin	Balmorhea	Laredo		
23%	16%	16%	3.4%	3.2%