The invasion autecology of *Iris pseudacorus* L. (Iridaceae) in South Africa

THESIS

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Abstract

Iris pseudacorus L. (Iridaceae) is an emergent aquatic macrophyte originating from Europe, north Africa, and western Asia, and is becoming an increasingly problematic invader in South Africa. By forming dense rhizomatic mats in the absence of natural enemies, *I. pseudacorus* outcompetes co-occurring indigenous biota, causing serious environmental and socio-economic challenges. *Iris pseudacorus* is a declared invader in South Africa, Argentina, New Zealand, the United States of America, and Canada, but little information is known regarding the species' invasive potential, particularly in the southern hemisphere, hindering the effectiveness of control efforts.

This study addresses this knowledge gap in a South African context, providing valuable insight into the invasion autecology of *I. pseudacorus* in South Africa. For effective management and control of *I. pseudacorus* in South Africa and the global south, its distribution and invasive potential must be determined, and its population genetics understood. Hence, this study aimed to map the current confirmed distribution of *I. pseudacorus* populations in South Africa, investigating the relative abundance of *I. pseudacorus* individuals in each population, and comparing their sexual reproductive outputs. Moreover, this study assessed the competitive interactions between *I. pseudacorus* and co-occurring native species *T. capensis,* and examined the genetic diversity present between and within South African *I. pseudacorus* populations.

Through field surveys, *I. pseudacorus* infestations were confirmed in eight of the country's nine provinces, with the highest number of infestations recorded in the urban hubs, and greatest population abundances reported in the warmer, wetter regions of South Africa. These surveys indicated that South African *I. pseudacorus* populations have enhanced their sexual reproductive output relative to native range populations, and a germination rate of ~ 83 % was determined in the laboratory. The results of a common garden competition experiment indicated that *T. capensis* may be a superior competitor over *I. pseudacorus*, but this was not supported by field observations, and may be a result of the short duration of the experiment. Using inter-simple sequence repeats (ISSRs), high genetic diversity was observed within and between populations of *I. pseudacorus*, indicating the employment of sexual reproductive strategies, and providing evidence for gene-flow between and within populations. Moreover, a weak negative correlation was observed between geographic distance and genetic similarity, indicating a largely anthropogenic spread of *I. pseudacorus*, and suggesting the occurrence of fewer founding events than reported in the United States.

This study provides useful insight into the invasion autecology of *I. pseudacorus* in South Africa, contributing to the ongoing research surrounding *I. pseudacorus* invasions worldwide, particularly in the southern hemisphere. These results contribute to the development of appropriate adaptive and integrated management strategies to control *I. pseudacorus* invasions in South Africa, and should be implemented before South African *I. pseudacorus* infestations reach the severity observed elsewhere.

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Chapter 1: General introduction

1.1 Introduction

Defined as species which establish, proliferate, spread, and persist in new geographic or climatic ranges, invasive alien species (IAS) pose serious threats to biodiversity and ecosystem functioning worldwide (Bright, 1999; Mack et al., 2000; Mullin et al., 2000). By altering processes integral to the productivity and effective functioning of an ecosystem, including primary productivity, nutrient cycling, disturbance regimes and hydrology, IAS can cause fundamental changes to ecosystem structure and functionality, leading to further environmental, economic, and social challenges (Sakai et al., 2001; Shackleton et al., 2007). For these reasons, IAS have been acknowledged as a main contributor to human-induced global change (Vitousek et al., 1997; Sakai et al., 2001), and their effects on the environment, the economy and society are becoming increasingly acknowledged.

1.1.1 Environmental and Ecological Impacts

The direct effects of IAS on ecological systems are relatively well understood. Species invasions can threaten the integrity of an ecosystem in a multitude of ways, many of which result in the break-down of ecological networks crucial to the functioning and productivity of the ecosystem in question (Mack et al., 2000; Vilà et al., 2011).

As invasive alien plants (IAPs) are often introduced to regions geographically isolated from their indigenous range, these invasive populations are likely to 'escape' from the suppression and population regulation typically provided by a suite of natural enemies (Keane and Crawley, 2002). Without such regulation, and with the reduced requirement for defence, IAPs are able to reallocate their resources to growth and propagation, providing them with a competitive edge over co-occurring indigenous plants which are still experiencing suppression from natural enemies (Crawley, 1987; Blossey and Notzold, 1995; Keane and Crawley, 2002; Callaway and Ridenour, 2004). As such, IAPs are often found to outcompete or displace indigenous species, altering or eliminating their long-lived interactions with native biota (Mack et al., 2002; Sunny et al., 2015). As ecological networks are complex, and by no means linear, these disturbances are likely to trickle down further into the network, causing further disruptions to integral interactions, such as those mutualistic interactions and networks between plants and their pollinators or seed dispersers (Pyšek et al., 2011). Disruptions to these networks can lead to drastic reductions in the richness, abundance, and diversity of indigenous species, reducing the system's bio- and functional diversity, and ultimately reducing the system's resilience to further disturbance (Peterson et al., 1998).

The integrity of ecosystems and the biodiversity therein can also be affected by the genetic introgression of native and alien species (Vilà et al., 2000; Crooks, 2002). Successful and prolonged hybridization between an alien and a native species can lead to reductions in the frequency of distinct native genotypes, leaving the community with little genetic variation, and diminishing the persistence and genetic integrity of the native species – potentially leading to its extinction (Huxel, 1999; Vilà et al., 2000). As is the case with direct competition mentioned above, the indirect competition occurring between alien and native species (with regard to genetic introgression and loss of native genotypes) can lead to higher-order effects within ecological networks (Olden et al., 2004). With increasing homogeneity of biota and the associated declines in bio- and functional diversity, it is likely that genetic introgression could lead to a decline in ecosystem or community resilience, and an increase in the ecosystem's susceptibility to further disturbance and invasion (Olden et al., 2004).

Invasive species further impinge on the effective functioning of an ecosystem by altering the various cycles upon which ecosystem functionality depends (Bright, 1999; Vilà et al., 2011). By altering established nutrient and hydrological cycles, for example, invasive species can alter community and population structure, as resource allocations will shift, leading to changes in biotic interactions such as competition, pollination, and herbivory (Vilà et al., 2011).

1.1.2 Economic and Societal Impacts

The socio-economic impacts of invasive species have been relatively well documented (Shackleton et al., 2007). The detrimental effects these species have on the provision of ecosystem goods and services, and on the economic enterprises which rely on such provisions, are both plentiful and relatively cosmopolitan (Shackleton et al., 2007). By reducing the productivity and functionality of an ecosystem, IAS hinder on the ecosystem's ability to provide the goods and services upon which society and economic activity depends (Pejchar and Mooney, 2009).

While it is undeniable that invasive species can lead to substantial economic challenges, it has proven difficult to capture the full cost associated with species invasions, and to quantify these costs (particularly the more abstract of which) in monetary terms (Pejchar and Mooney, 2009). Thus, it is likely that the economic losses associated with invasive species are often reported conservatively, and that the economic impact of these species is underestimated. However, while it is challenging to accurately quantify the full cost associated with IAS and their effects on ecosystem service provision, such measures (although likely conservative estimates) prove useful in communicating the inherent value of healthy, functioning ecosystems and the biodiversity therein to politicians, policy makers and the general public (Gómez-Baggethun and Ruiz-Pérez, 2011).

Although the economic costs associated with a decline in the provision of ecosystem services are somewhat abstract and difficult to determine accurately, the direct costs associated with the management and control of IAS are substantially more straightforward. Managing and controlling IAS is necessary to mitigate the negative environmental, economic, and social impacts associated with species invasions. At present, there are three overriding control methods commonly employed to manage, remove, and regulate IAS worldwide, namely: i) mechanical control; ii) chemical control; and iii) biological control.

'Mechanical control' refers to the process of manually removing the invader from the system of interest. This process is fairly straightforward and low-risk, but is often very costly and time-consuming (Núñez-González et al., 2021). 'Chemical control' involves the use of pesticides to control the invader. As is the case for mechanical control, chemical control provides quick results, and its application is quite straightforward. However, there are risks involved with the use of pesticides to control invaders, as pesticides are not necessarily species-specific, and so their effects are often felt not only by the target species, but by the co-occurring indigenous biota (Kettenring and Adams, 2011; Núñez-González et al., 2021). Lastly, 'biological control' refers to the use of biological organisms (herbivores, parasites, etc.) to control species invasions. Biological control is less straightforward than both mechanical and chemical control, and results may take years to materialize. However, this method often proves to be relatively cost-effective and economically sustainable (McConnachie et al., 2003; van Wilgen and De Lange, 2011). While all three control methods have proven to be effective, their relative success is context- and species-specific.

1.1.3 IAS in freshwater ecosystems in South Africa

Freshwater ecosystems in South Africa are prone to invasion by aquatic macrophytes (Hill et al., 2020). Invasive aquatic macrophytes often form dense monotypic stands which can alter the system's hydrology, utility, biodiversity, and ecological functionality (Hill et al., 2020). The negative impacts of floating aquatic invaders on the economy and the environment have been well documented (Hill 2003; Coetzee et al., 2011a; Hill and Coetzee 2017). Submerged and emergent aquatic invaders have, however, received substantially less attention. Coetzee et al. (2011b) suggest that the lack of attention given to these plants may be due to a variety of factors, including the possibility that their invasions have been weakened by competition from indigenous submerged and emergent plants, or from indigenous and/or invasive floating plant species. When in high abundance, floating aquatic macrophytes limit the amount of light entering a water body, reducing the amount of primary productivity occurring and resulting in anoxic conditions detrimental to submerged and emergent macrophytes growing below the water's surface (Scheffer and van Nes, 2007). However, with the successful control of many of South Africa's floating invaders, new niches may be provided in which submerged and emergent alien macrophytes are able to invade (Scheffer and van Nes, 2007; Coetzee et al., 2011b), forming the topic of this thesis.

1.2 The Invasion Process

The risk of introducing alien species to novel environments has increased substantially in recent decades as a result of globalisation and the accompanied increases in travel and trade (Roy, 1990; Mullin et al., 2000; Keane and Crawley, 2002). As globalisation is continuing to intensify and increased opportunities for introduction events are becoming available, it is likely that we will observe a substantial increase in the number of species invasions occurring in the near future (Hui and Richardson, 2016; Hinz et al., 2019).

Species invasions occur in several stages. Blackburn et al. (2011) propose a unified framework for biological invasions, in which these stages are separated by various barriers (Figure 1.1). First, the alien species and their propagules must overcome geographical barriers and be transported to a novel environment. In this environment, the introduced species faces further challenges to survival, reproduction, and establishment (García-Ramos and Rodríguez, 2002; Davis, 2009; Blackburn et al., 2011; Pearson et al., 2018). The established species must then be able to spread, proliferate or disperse further into their new environment, often to the detriment of the established indigenous species with which the aliens will interact (Davis, 2009; Blackburn et al., 2011). Although a vast number of species are introduced to regions outside of their native ranges each year, only a small portion of these species are able to establish viable populations, and even fewer proceed to become invasive. Furthermore, many species capable of establishing in novel environments experience substantial time-lags between their introductions and their subsequent invasions (Blackburn et al., 2011; Rouget et al., 2015; Hinz et al., 2019). Whether the introduced alien species will become invasive or not depends on a variety of factors, including the biology of the potential invader, the abiotic characteristics of the invaded landscape, and the biotic interactions occurring therein (Vilà and Weiner, 2004; Pearson et al., 2018). Understanding how alien species invade landscapes is crucial to controlling their invasions and minimising the impacts thereof (Keane and Crawley, 2002).

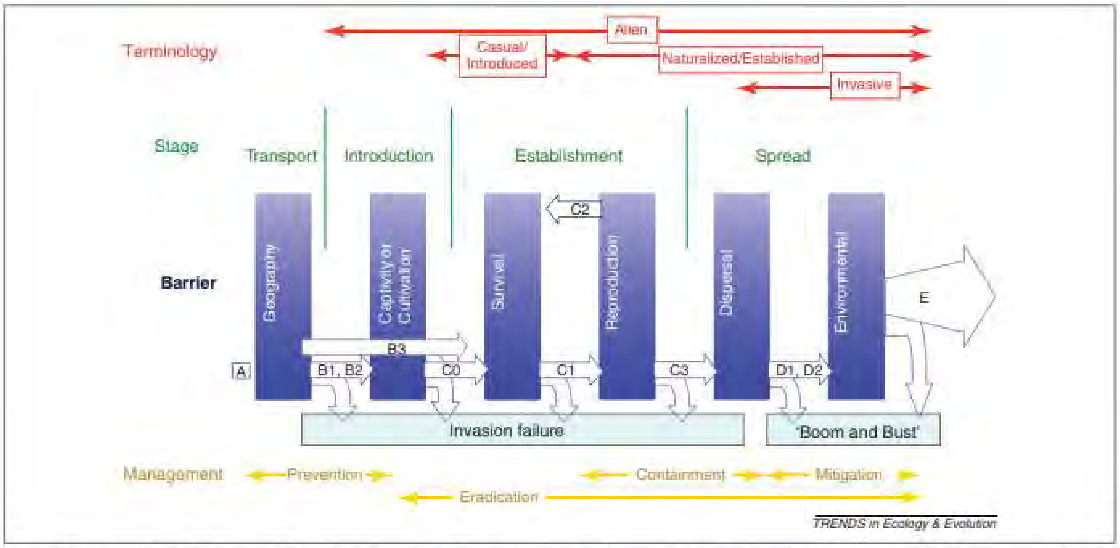


Figure 1. The proposed unified framework for biological invasions. The proposed framework recognises that the invasion process can be divided into a series of stages, that in each stage there are barriers that need to be overcome for a species or population to pass on to the next stage, that species are referred to by different terms in the terminology depending on where in the invasion process they have reached, and that different management interventions apply at different stages. Different parts of this framework emphasise views of invasions that focus on individual, population, process, or species. The unfilled block arrows describe the movement of species along the invasion framework with respect to the barriers, and the alphanumeric codes associated with the arrows relate to the categorisation of species with respect to the invasion pathway given in Table 1 (main text).

Figure 1.1: The proposed framework for biological invasions (Blackburn et al., 2011)

1.2.1 Introductions

The process of alien species introduction is fairly straightforward. Alien species are introduced to regions outside of their native ranges (i.e. those in which they evolved) either intentionally, with the aim of the species serving a functional purpose in its introduced range – such as for agriculture, restoration, recreation or for ornamental purposes – or accidentally, whereby the species "tag along" with the transport of goods and/or people, or are forced to expand their ranges due to change or disturbance in their native landscapes (Williamson, 1996; Bright, 1999; Lowe et al., 2000; Pimentel et al., 2005; Hui and Richardson, 2016). Less straightforward, however, are the processes involved in species establishment, dispersal, and ultimately invasion.

1.2.2 Establishment and Spread

The likelihood that an introduced alien species will successfully establish and spread in its introduced range depends largely on the species' evolution and life history characteristics (Sakai et al., 2001; Pearson et al., 2018). For example, species which have evolved traits that allow for rapid proliferation and phenotypic plasticity amongst propagules are perhaps more likely to become successful invaders than those which have not (Sakai et al., 2001). Therefore, the study of such traits ought to be included when assessing the invasion potential of a species or population, or when attempting to predict their invasiveness in a particular system. With regard to invasive plant species, the likelihood of successful establishment and spread depends largely on the species' reproductive or propagative abilities, growth rates, time taken to reach reproductive maturity, ability to adapt to stress and/or disturbance, and the nature of the biotic and abiotic interactions experienced by the species in the invaded system (Sakai et al., 2001; Vilà and Weiner, 2004; Barrett et al., 2008; Gaskin et al., 2016).

1.2.2.1 Reproduction and Propagation

As the success of invasions by alien plants depends largely on their ability to reproduce and propagate effectively, it is important to understand the primary mode of reproduction in the species of interest (Barrett et al., 2008; Gaskin et al., 2016). Plant reproductive strategies vary considerably both between and within plant taxa, with angiosperms demonstrating a particularly high degree of diversity (Barrett et al., 2008). Angiosperms are often able to reproduce both sexually and asexually, through means of outcrossing and selfing, and with or without the aid of a pollinator (Whitton et al., 2008). However, the relative importance and employment of such strategies varies considerably and could differ both between and within species or populations.

Aquatic plants have been known to reproduce primarily through clonal, or vegetative, propagation as opposed to sexual modes of reproduction (Johansson and Nilsson, 1993). Although many of these species produce viable seed and are able to reproduce sexually, studies have suggested that the dominant reproductive mechanism employed by these plants tends to be clonal, or vegetative (Barrett, 1980). While the dispersal of vegetative propagules is restricted in terrestrial environments, aquatic environments support their dispersal, and the additional energy resources available to vegetative propagules (as opposed to seeds) may have led to their predominance in aquatic plant communities (Silvertown, 2008; Gaskin et al., 2016). Clonal reproduction and the effective dispersal of vegetative propagules of invasive aquatic plant species carries the additional benefit of colonization and population establishment in areas where viable sexual partners or pollinators may be unavailable.

1.2.2.2 Biotic interactions

Many successful invasions by alien plant species have been attributed to the mechanisms proposed in the Enemy Release Hypothesis (ERH) (Keane and Crawley, 2002). The ERH suggests that invasive plants introduced to novel environments experience substantial declines in herbivory by natural enemies, allowing their populations to increase at faster rates (Keane and Crawley, 2002). The decline in herbivory experienced by invasive plants may support an evolutionary change through which the plants' relative resource allocations shift (Joshi and Vrieling, 2005). In the absence of natural enemies, invasive plants may not need to invest such large amounts of resources into anti-herbivory defences, and can rather allocate these resources to growth, reproduction, and competition (Joshi and Vrieling, 2005). This idea has been postulated in the Evolution of Improved Competitive Ability (EICA) hypothesis, but direct evidence to support this hypothesis has been limited (Joshi and Vrieling, 2005).

It is predicted by Joshi and Vrieling (2005), who combine the ERH and EICA hypotheses, that plants introduced into environments without specialist herbivores may evolve in such a way that their defence strategy shifts, reducing the production of resource-expensive defences aimed at specialist herbivores, and increasing the production of cheaper defences aimed at generalist herbivores. This shift in defence strategy would allow for an increased availability of resources to be allocated elsewhere, such as to growth, competition, and reproduction, potentially providing the invader with a competitive advantage over co-occurring native species (Joshi and Vrieling, 2005). In cases where the ERH holds true, biological control may be an effective means of controlling plant invasions, as the plant would be reintroduced to its natural enemies, and thus lose out on the advantages posed in the ERH.

The biotic resistance hypothesis suggests that the biotic diversity of an ecosystem influences its susceptibility to invasion, and that ecosystems with low biotic diversity are more susceptible to invasion than those with high biotic diversity (Levine and D'Antonio, 1999). Ecosystems with low diversity provide more opportunity for invasion as there is a greater "niche space" available than is the case for ecosystems with high diversity (Levine and D'Antonio, 1999). Ecosystems with greater species diversity may house more robust interaction networks than those with low diversity, providing more resilience to invasions by introduced species through higher levels of both competition and consequent niche-occupation (Levine and D'Antonio, 1999). As interactions play a large role in determining the outcome of invasions, it is important to consider such interactions when managing species invasions, and in the implementation of effective control strategies.

1.3 Study Species: *Iris pseudacorus* (Linnaeus, 1753) (Iridaceae)

One of the more recently recognised aquatic invaders in South Africa is *Iris pseudacorus* (Linnaeus, 1953) (Iridaceae), commonly known as Yellow flag iris. *Iris pseudacorus* is a relatively new invader in South Africa, with its first naturalised population recorded in 2004 (Jaca and Mkhize, 2015). While *I. pseudacorus* may not be amongst the country's most problematic invaders for the time being, the severity of its invasions elsewhere, including the United States, New Zealand and Argentina, emphasise the importance of monitoring, managing and ultimately controlling South Africa's *I. pseudacorus* invasions as early as possible.

1.3.1 Plant Morphology

The genus *Iris* comprises over 250 species and is widely distributed in the northern temperate zones (Jaca and Mkhize, 2015). The genus has its centre of diversity in Asia and contains no species native to southern Africa (Jaca and Mkhize, 2015).

Iris pseudacorus (Yellow flag) is a perennial aquatic angiosperm with showy yellow flowers (approximately 8-10 cm in diameter) (Figure 1.2) and an erect growth form (Sutherland, 1990). The leaves of *I. pseudacorus* are long and ensiform in shape, taking on a fan-shape where they emerge from the ground. The plant's rhizomes have diameters of approximately 1-4 cm, with roots growing between 10-30 cm in length (Sutherland, 1990; Jacobs et al., 2010). The rhizomes of *I. pseudacorus* plants are able to form dense, sometimes impenetrable stands (Figure 1.3) on the banks of waterbodies, which limit the growth and spread of neighbouring plants, drastically reducing native plant diversity (Thomas, 1980; Jacobs et al., 2010; Jaca and Mkhize, 2015).

Figure 1.2: *Iris pseudacorus* flower with bee visitor (photo by E. Sandenbergh, 2019)

Figure 1.3: Dried *Iris pseudacorus* rhizome mat, Emmarentia Botanical Gardens, Johannesburg (photo by E. Sandenbergh, 2019)

1.3.2 Invasion Biology

Iris pseudacorus is native to North Africa and Eurasia, and is distributed widely between North Africa and Siberia (Morgan, 2010; Jaca and Mkhize, 2015 Gervazoni et al., 2020; Grewell et al., 2021; Minuti et al., 2022) (Figure 1.4). *Iris pseudacorus* escaped its natural range, and has been declared invasive in the United States, Canada, New Zealand, Argentina, and South Africa (Figure 1.4), but the degree to which it has invaded differs greatly (Jaca and Mkhize, 2015; Coetzee, 2019, pers. comms.; Gervazoni et al., 2020). South Africa's first record of *I. pseudacorus* occurring outside of cultivation was noted in 2004, where it was growing along the Gauteng Province's Vaal River (Jaca and Mkhize, 2015). It is likely that *I. pseudacorus* was introduced outside of its native range as an ornamental plant, but it has proceeded to invade the country's water systems effectively enough for it to be classified as a category 1a invasive plant species under the National Environmental Management: Biodiversity Act (NEMBA) of 2004 (Jaca, 2013; Department of Environmental Affairs, 2016).

Figure 1.4: Global distribution of *Iris pseudacorus*, adapted from the Global Biodiversity Information Facility (GBIF, 2021)

As a category 1a invader, *I. pseudacorus* populations are required to be removed, destroyed, and eradicated, and cannot legally be cultivated. At present, *I. pseudacorus* populations in South Africa are being controlled both mechanically and chemically. While these control methods may prove effective in controlling small, isolated populations of *I. pseudacorus*, larger and more dense populations of the weed may not be so easily eradicated. Mechanically controlling dense populations of *I. pseudacorus* can prove challenging as the plants form dense rhizomatic mats which can be difficult to penetrate, and failure to remove all rhizome fragments will likely result in their regrowth, thus requiring further control. Chemical control may prove effective in reducing the densities of weed monocultures, or eradicating stands of the weed where it has invaded heavily. However, as there is no herbicide specific to *I. pseudacorus*, the effects of the herbicide will likely trickle down into the system, potentially harming native biota.

Iris pseudacorus is capable of reproducing both sexually and asexually, spreading via both seed dispersal and vegetative propagation (Sutherland, 1990). When reproducing sexually, *I. pseudacorus* is an obligate outcrosser, with each plant producing a vast number (400-1,500) of smooth, reddish to dark brown seeds (depending on maturity) which are flat and circular in shape, with hard seed coats (Figure 1.5). The seeds produced have a high level of buoyancy to allow for dispersal by water, and high levels of both seed viability and germinative power have been reported in *I. pseudacorus* populations in the northern hemisphere (Coops and van der Velde, 1995; Gaskin et al., 2016; Mopper et al., 2016; Rosbakh et al., 2020; Gillard et al., 2021). However, quantitative data on the viability and germinability of *I. pseudacorus* seeds is scarce, particularly in the southern hemisphere. As both biotic and abiotic characteristics differ notably within and between the native and invaded ranges, the results obtained for *I. pseudacorus* populations in the global north cannot necessarily be inferred onto those in the global south.

Figure 1.5: *Iris pseudacorus* seeds, a) released from dehisced seed capsules, Kurper Oord, Hartbeespoort Dam (photo by J. Coetzee, 2020); and b) germinating within recently dehisced seed capsule, Glenhazel Park, Johannesburg (photo by E. Sandenbergh, 2020)

1.4 Problem statement

Very little information exists on the invasion biology and autecology of *I. pseudacorus* in the global south. As *I. pseudacorus* populations may behave differently in the southern hemisphere as a result of different conditions, this research gap may hinder the effectiveness of control programmes aimed to reduce or mitigate the effects of the weed. To allow for the most effective control strategies to be implemented against *I. pseudacorus* invasions in the global south, it is important that research efforts are put in place to address this knowledge gap. This thesis will form part of such an effort, by addressing aspects of the invasion biology and ecology of *I. pseudacorus* populations in South Africa. Similar studies are being conducted by collaborators in Argentina and New Zealand, forming a global south initiative to tackle this weed. To effectively monitor, manage and control *I. pseudacorus* populations in South Africa and the global south, their distributions must be known, their invasive potentials determined, and their population genetics understood.

1.4.1 Aims and Objectives

This study aimed to better understand the invasion biology and autecology of *I. pseudacorus* populations in South Africa. This information could better equip invasion control organisations to manage and control *I. pseudacorus* invasions while in their infancy, mitigating the harmful effects of a severe invasion.

Firstly, this study assessed the current distribution of *I. pseudacorus* populations in South Africa, reporting on the abundance and sexual reproductive output of these populations, and investigating the potential effects of climate on distribution, abundance, and reproductive potential. It is expected that *I. pseudacorus* will occur across South Africa's temperate and subtropical regions, with the greatest population abundance and highest reproductive outputs in regions with high temperatures and high levels of precipitation. Invasive potential was further investigated by evaluating the competitive ability of *I. pseudacorus* when grown in combination with native *Typha capensis*.

To further address the main aim of this study, the population genetics of South African *I. pseudacorus* populations was investigated. By assessing the degree of genetic diversity present between and within populations, insight is provided into the relative employment of sexual vs asexual reproductive strategies by South African *I. pseudacorus* populations. A high level of genetic diversity has been observed in invasive *I. pseudacorus* populations in the United States (Gaskin et al., 2016), and so it is expected that this will hold true for South African *I. pseudacorus* populations.

1.4.2 Thesis Layout

This thesis comprises four chapters. Chapter 1 reviews the relevant literature, providing the background information necessary to justify conducting this study. Chapter 2 presents the distribution and abundance data for *I. pseudacorus* populations in South Africa, including their competitive abilities and respective reproductive potentials. Chapter 3 investigates the population genetics of South African *I. pseudacorus* populations, evaluating the degree of genetic diversity present, and assessing their primary mode of reproduction. Finally, Chapter 4 discusses and integrates the results obtained in Chapters 2 and 3, providing a consolidation of the information gained throughout this study, identifying the persisting research gaps, and addressing the key findings and implications of this study for the control of *I. pseudacorus* populations in South Africa and the global south.

Chapter 2: Distribution and invasive potential of *Iris pseudacorus* populations in South Africa

2.1 Introduction

2.1.1 Species distributions

Species distributions are influenced by a multitude of biotic and abiotic factors. The abiotic factors known to influence species distributions, particularly those of plant species, include climatic factors (such as temperature, humidity, and sunlight), edaphic factors (those pertaining to soil, including soil type, pH, water retention and nutrient content), and social factors (such as land-use). Plant distributions are also influenced by biotic factors – the interactions they experience with other biota – including, amongst others, competitive interactions between plants, plant-pollinator interactions, plant-herbivore interactions, and the microbial interactions occurring below-ground.

2.1.1.1 Plant species distributions

For a species to establish in an area, the abiotic components of the environment must allow for the species' survival and reproduction (Baker et al., 2000). That is, the area must be climatically and edaphically suitable for the plant to grow and spread. This is referred to as the fundamental niche of the species (where the species could, but does not necessarily, occur), and does not consider the biotic interactions occurring in the system. As biotic interactions hold influence in determining species distribution patterns, it is important to consider the species' realized niche (where it does occur) once its fundamental niche has been identified. This has been illustrated and discussed in studies and reviews critiquing the use of climatic models alone when attempting to map species distributions, including Davis et al. (1998a, b) and Lawton (2000). While biotic interactions cannot be ignored when determining species distribution patterns, the use of climate indices to determine and map species distributions has achieved greater successes when utilised for plant taxa than is the case for animal taxa (Woodward, 1987; Baker et al., 2000).

Plant taxa respond differently to changes to their biotic and abiotic conditions, with various factors affecting their ranges in different ways. For example, with all other variables remaining constant, the range of a drought-tolerant plant species will not be affected by changes in water availability to the same extent as a plant with limited drought-tolerance, and thus the plants' ranges would not expand or contract in the same manner to this environmental perturbation. However, it is very rare that the distribution pattern of a species is influenced solely by a single climatic variable – for example, drought (Baker et al., 2000). As it is most often a wide array of interconnected and interacting variables that result in the species distribution patterns observed, a deeper understanding of the biology and ecology of the species in question would assist in predicting future distributional patterns. Understanding species distributions can also aid our understanding of their biologies and ecologies; why does the species occur here but not there? What factors would allow for species proliferation in certain areas, and the restriction of their ranges in others? In an invasion biology context, this information would be particularly valuable, as it would allow for invasion control organisations to prioritise high-risk systems, and conduct their control efforts in the most efficient manner.

2.1.1.2 Aquatic plant distributions

In addition to the aforementioned factors influencing species distribution patterns, several additional factors hold influence when considering the distributions of aquatic plant species. These factors include, amongst others, water quality, hydrology, the connectivity of water systems, and sedimentation, which affect floating, submerged, and emergent aquatic macrophytes alike, albeit to differing degrees.

Water quality largely refers to the physico-chemical properties of the waterbody, including nutrient loading and content, pH, salinity, dissolved oxygen, the presence of toxicants, etc. Aquatic plant species often have very different tolerances to the perturbations in water physico-chemistry, but a relatively uniform response from aquatic macrophytes is to increase their growth in response to increasing resource availability (Davis et al., 2000).

This holds true for invasive aquatic macrophytes, which capitalise on increased resource availability in the systems they invade. As suggested in the theory of fluctuating resource availability, an increase in the amount of unutilised resources increases the community's susceptibility to invasion (Davis et al., 2000). An increase in resource availability reduces the need for competition, allowing an increase in resource allocation to growth, survival, and reproduction. This may benefit invasive species to a greater degree than native species, which would still be required to allocate a portion of resources to anti-herbivory defences (Keane and Crawley, 2002).

2.1.2 Invasive potential: Reproduction

The invasiveness of a plant species depends largely on its ability to reproduce and propagate effectively in its new environment (Barrett et al., 2008; Gaskin et al., 2016). The rate at which propagules are produced and dispersed has a significant impact on the population's invasiveness, or its ability to spread into and become established and abundant in novel environments (Coops and van der Velde, 1995; Colautti et al., 2006). Understanding the mechanisms involved in the establishment and spread of invasive plant species is an important first step in monitoring and managing current invasions, predicting the nature of future invasions, and ultimately limiting the invasiveness or invasion potential of the species or population of interest (Barrett et al., 2008).

A population's ability to spread into and become established in novel environments is largely dependent on propagule pressure, or the rate at which propagules, including seeds and vegetative fragments, are released or introduced (Williamson, 1996; Colautii et al., 2006). 'Propagule pressure' is a combined measure of both propagule size – the number of individual propagules released during a single event, e.g. during budburst – and propagule number – the number of separate events through which propagules are released (Lockwood et al., 2005). As the rate of propagule production and release increases, so too does propagule pressure, resulting in increased opportunities for the spread and establishment of invasive plant species (Lockwood et al., 2005; Colautti et al., 2006).

In general, invasive plant populations with high rates of propagule production and release are likely to succeed in their invasions, as the opportunities for both introduction and establishment are enhanced (Colautti et al., 2006; Lockwood et al., 2009). Therefore, invasive plant populations with high seed outputs have a greater invasive potential than those with low seed outputs (Lockwood et al., 2009), enabling them to invade new landscapes quite rapidly. However, if the population does not produce viable seed, its seed output would be irrelevant in determining its invasive potential as the seeds produced would not be capable of germination. If an already-invasive plant population is found to produce large numbers of unviable seed, it is likely that the population spreads clonally through the production of vegetative fragments rather than through the production and dispersal of sexually produced seed. This is common with invasive weeds, to which clonal reproduction could prove highly advantageous during colonisation or range-expansion events (Baker, 1955). Alternatively, if an invasive population is found to produce high numbers of viable seed, it is likely that the population either reproduces predominantly or partially through sexual means of reproduction.

Seed production in *I. pseudacorus* plants can be very high (between 400-1,500 seeds per plant), with high levels of seed dispersal, viability and germinative power (Coops and van der Velde, 1995; Gaskin et al., 2016; Mopper et al., 2016). As *I. pseudacorus* occurs across a range of habitats and climate zones (Gervazoni et al., 2020; Grewell et al., 2021; Minuti et al., 2022), it is likely that exposure to different climatic, environmental, and ecological conditions would affect the reproductive output of the species. As seed production, viability and germinative power all influence the invasiveness of a sexually reproducing plant species (Coops and van der Velde, 1995), investigating these aspects of *I. pseudacorus* reproduction in a South African and southern hemisphere context is an important step in understanding its population dynamics in the global south, managing these populations, and ultimately reducing the potential for further invasion.

2.1.3 Invasive potential: Competition

While the likelihood of an alien plant species spreading into and establishing in new environments is largely influenced by their ability to reproduce and propagate effectively, the likelihood of the species becoming invasive depends largely on the nature of its interactions with established biota. Crawley (1990) suggests that interspecific competition may be amongst the most important factors in determining the likelihood of a successful invasion, and ultimately in determining the dynamics of plant communities. Invasive plants introduced to new environments will compete with the established native and invasive species for resources including water, light, nutrients, and space (Vilà and Weiner, 2004). If the invasive species is better equipped at acquiring the limiting resources in its new environment and is found to be a better competitor than its native or invasive counterparts, drastic changes in species composition could occur, with a decline in the abundance of the previously established species and an increase in that of the introduced invader. Thus, it is likely that an alien species with a high competitive ability will invade successfully when established in environments with less competitive counterparts (Baker, 1955). Alternatively, an alien species which does not have a competitive advantage in its new environment will be unlikely to succeed in its invasion (Keane and Crawley, 2002).

Many successful invasions by alien plant species have been attributed to the mechanisms proposed in the Enemy Release Hypothesis (ERH) (Keane and Crawley, 2002). With a prolonged absence of herbivory, invasive plants lose the need to invest large portions of their resources into anti-herbivory defences, making these resources available for reallocation to growth and enhanced competitive ability (Joshi and Vrieling, 2005). This may provide the invasive species with a competitive edge, increasing the likelihood that the invader will outcompete the native species, altering the structure and dynamics of the communities and systems in question.

2.1.4 Anthropogenic influences

While plant species distributions are influenced heavily by environmental and ecological factors, an increasingly important factor to consider is the role of anthropogenic activity. This is particularly important when considering invasive plant species, which are often spread (both directly and indirectly) as a result of human activity (Martin and Coetzee, 2011).

Anthropogenic activity, including travel and trade, is often responsible for the introduction and spread of invasive species. Furthermore, human-induced environmental disturbance and degradation may facilitate invasions by reducing competition from native species (including soil pathogens), thus providing new niches for invasive species to occupy (Hierro et al., 2006). For many invasive plant species, such disturbances are required for their invasions to be successful, as they are able to establish and proliferate in the absence of (or with a reduction in) competition, and thus an increase in resource availability (Hierro et al., 2006; Lear et al., 2020).

Invasive species which do not require disturbance to establish and spread, but which rather induce disturbance, can be referred to as “drivers” of environmental degradation (MacDougall and Turkington, 2005). The species which then benefit from such disturbance (that induced by previous invasions, as well as that caused by eutrophication and habitat destruction, for example) are “passengers” and would not succeed in their invasions in the absence of previous or existing disturbance (MacDougall and Turkington, 2005). When these species further add to environmental and ecological degradation or destabilisation, they are deemed “back-seat drivers” of environmental disturbance (Bauer, 2012). Many invasive aquatic plants are either “passengers” or “back-seat drivers” and require human-mediated disturbance to become successful in their invasions (MacDougall and Turkington, 2005; Bauer, 2012).

While the introduction and spread of invasive species is often unintentional, human activity is largely responsible for many plant species invasions. The aquarium trade has been recognised as a main contributor to the introduction and spread of submerged aquatic macrophytes (Martin and Coetzee, 2011), and the horticultural industry plays a large role in the introduction and spread of many aquatic macrophytes (Maki and Galatowitsch, 2004), as appears to be the case with the introduction and spread of *I. pseudacorus* in South Africa (Jaca and Mkhize, 2015).

As a result, distribution patterns of many invasive angiosperms appear to be clumped in and around urban hubs (Wania et al., 2006; Kühn et al., 2017), which ought to be considered when mapping and modelling such distributions, so as to avoid assigning relevance to certain environmental or ecological factors prevalent in the area of interest.

2.1.5 *Iris* distribution

The genus *Iris* (Iridaceae) comprises over 250 species, and is widely distributed in the northern temperate zones, with its centre of diversity in Asia (Jaca and Mkhize, 2015). While approximately 1036 species of the Iridaceae family occur in southern Africa, since the retreatment of *Morea* species into their own genus, there are no representatives of the genus *Iris* native to the region (Jaca and Mkhize, 2015).

2.1.5.1 *Iris pseudacorus* distribution

The native range of *I. pseudacorus* includes Britain, Europe, the Mediterranean region, Western Asia, and Northern Africa (Sutherland, 1990; Gervazoni et al., 2020; Grewell et al., 2021; Minuti et al., 2022). In its native range, *I. pseudacorus* occurs throughout a wide variety of environments and environmental conditions, but the species prefers humid temperate climates (Minuti et al., 2022). *Iris pseudacorus* is commonly associated with wetlands, marshes, bogs, and floodplains, and often occurs along the banks of rivers, streams, lakes, and ponds (Sutherland, 1990). Outside of its native range, *I. pseudacorus* has been reported in both inland and coastal wetlands across a variety of global ecozones (Gervazoni et al., 2020; Grewell et al., 2021).

As it is an emergent aquatic plant, *I. pseudacorus* distributions are restricted to areas with high soil water content, but the species can occur on a wide range of soil types, including anoxic soils and those with a pH ranging from 3.6 to 7.7 but prefers soils that are neutral or slightly alkaline (Sutherland, 1990; Engin et al., 1998; Yousefi and Mohseni-Bandpei, 2010). While *I. pseudacorus* most often occurs in freshwater environments, it is also able to survive in saline or brackish waters (Sutherland, 1990; Engin et al., 1998; Gillard et al., 2021). Although the species prefers wetter soils, it is able to survive periods of drought, and its rhizomes have been shown to continue growing after being exposed to drought conditions for a period of three months (Sutherland, 1990; Yousefi and Mohseni-Bandpei, 2010). As well as preferring moist soils, *I. pseudacorus* grows best under high nutrient (or eutrophic) conditions, particularly with regard to soil nitrogen content, which strongly influences the plant's growth (Ellenberg, 1979; Sutherland, 1990; Leng et al., 2009; Morgan et al., 2020).

As South Africa's waters are considered amongst the most eutrophic in the world, aquatic macrophytes capitalise on the nutrient-rich waters, with their growth no longer limited by nutrient uptake (Burke and Grime, 1996; Smith et al., 1999; van Ginkel, 2011; Coetzee and Hill, 2012). As invasive aquatic plants, including *I. pseudacorus,* have been known to capitalise on increased resource availability in the waterbodies they invade, the high levels of nutrients in South Africa's eutrophic systems are likely to have increased their susceptibility to invasion by aquatic invaders (Daehler, 2003; Burns, 2004; Coetzee and Hill, 2012).

2.1.5.2 *Iris pseudacorus* distribution in South Africa

Iris pseudacorus was likely introduced outside of its native range as an ornamental pond plant due to its showy yellow flowers (Sutherland, 1990), and has since been declared invasive in Argentina, New Zealand, the United States of America, Canada, and South Africa (Gaskin et al., 2016; Minuti et al., 2021). *Iris pseudacorus* is a frequent invader of wetlands and stream banks, where it spreads from both rhizome fragmentation and seed production, forming dense, often impenetrable stands (Sutherland, 1990; Jaca and Mkhize, 2015).

The invasion of *I. pseudacorus* in South Africa is still in the "lag" phase (Blackburn et al., 2011), with the first record of the plant growing outside of cultivation made in 2004, where it was growing along the Vaal River (Jaca and Mkhize, 2015). At the time of publishing, Jaca and Mkhize (2015) reported 24 naturalised populations of yellow-flag iris in South Africa, in the Gauteng, Limpopo, Kwa-Zulu Natal and Western Cape Provinces. To date, many more records of *I. pseudacorus* have been reported, and the plant has been found to occur in almost every province in South Africa, with the exception of the arid Northern Cape. While some of these records are not necessarily those of invaded sites, but rather where the species has yet to 'escape' cultivation, the rigorous growth and spread of the species – even in its native range (Gaskin et al., 2016) – suggests that these populations ought to be included as they have the potential to spread further.

Iris pseudacorus populations in South Africa are most often found alongside those of the invasive *Pontederia cordata* L. (Pontederiaceae) and *Typha latifolia* L. (Typhaceae), as well as the native *Typha capensis* Rohrb. (Typhaceae). While these species are known to co-occur in South Africa's aquatic ecosystems, the competitive interactions occurring between them have not yet been investigated. Moreover, as the species are likely to have been introduced to the invaded systems at different times, it would be worthwhile to investigate the potential priority effects influencing the competitiveness of the species (Fukami, 2015).

Iris pseudacorus has been referred to as an "aggressive" competitor capable of engineering ecosystems, and drastically reducing native plant diversity (Thomas, 1980). Due to its aggressive growth and ability to form dense, compact stands of rhizomes, *I. pseudacorus* is able to spread quite rapidly, while preventing the establishment of other species unable to penetrate its dense rhizomatic mats (Dyer et al*.*, 1976; Thomas, 1980; Lamote et al*.*, 2002). As this rhizomatic mat can prevent establishment of other species, it is likely that *I. pseudacorus* would have a competitive edge over co-occurring species when it is introduced to a system first.

2.1.6 Aims and hypotheses

This chapter aimed to i) determine the current distribution and abundance of *I. pseudacorus* populations in South Africa; ii) investigate the potential climatic factors influencing *I. pseudacorus* distributions, densities and sexual reproductive outputs in South Africa; iii) determine the sexual reproductive outputs of *I. pseudacorus* populations in South Africa; and iv) investigate the competitive interactions occurring between *I. pseudacorus* and a co-occurring native plant species, *T. capensis*. This information will provide a better understanding of which regions or systems in South Africa are most at risk to further invasion by *I. pseudacorus.* Furthermore, this information will inform invasion control practitioners by identifying where control is needed most urgently, which abiotic factors to consider when modelling future *I. pseudacorus* distributions, and by identifying the high-risk regions or systems that ought to be monitored closely to prevent or restrict further invasions by *I. pseudacorus*.

It is hypothesised that i) *Iris pseudacorus* populations will have wider distributions and greater abundances in regions with warmer and more humid climates, as well as in major urban hubs; ii) temperature and precipitation will be the most significant abiotic factors influencing South African *I. pseudacorus* distributions, with warmer, wetter regions being more susceptible to invasion by *I. pseudacorus*; iii) the sexual reproductive output of South African *I. pseudacorus* populations will be high; and iv) *I. pseudacorus* will be the superior competitor when competing against the native *T. capensis*.

2.2 Materials and Methods

2.2.1 Distribution, abundance, and sexual reproductive potential

2.2.1.1 Fieldwork

Records of *I. pseudacorus* in South Africa were obtained from various sources, including the South African Plant Invasion Atlas (SAPIA), the South African National Biodiversity Institute (SANBI), and various Citizen Science and social media platforms, including iNaturalist, Facebook, and Twitter. These records were compiled, and the majority were ground-truthed during the plant's flowering season (September to November) in 2019, when a nation-wide survey was carried out (Table 2.1). The sites which were not ground-truthed during this nationwide survey were either inaccessible, on private property, or were not recorded in time to include in the surveys.

At each of the sites surveyed, the presence or absence of *I. pseudacorus* was recorded, and where present, three quadrat-surveys were conducted. For each 0.5 m x 0.5 m quadrat, the following plant parameters were recorded: number of leaves, number of reproductive stems, number of flowers, maximum height, and number of individual plants (incl. daughter plants). The nationwide surveys were conducted again using a subset of sites (see Table 2.1) in the plant's seeding season (February and March 2020), with the same parameters recorded, replacing number of flowers with number of seed capsules produced to determine the sexual reproductive output of the different populations. Ten capsules per site were collected and brought back to Rhodes University, where the number of seeds in each capsule was determined in the laboratory. The reproductive data obtained were used to determine the: i) mean number of reproductive stems produced per m^2; ii) mean number of flowers produced per reproductive stem, iii) mean number of seed capsules produced per reproductive stem; and iv) mean number of seeds produced per seed capsule.

Area cover (in m^2) was estimated at each site to be used alongside the plant parameters in determining the abundance of *I. pseudacorus* individuals at each site. At highly invaded sites, and sites at which area cover could not be determined on-site, polygons were created and measured using Google Earth Pro ver. 7.3 to obtain a more reliable estimate.

2.2.1.2 ArcGIS

Confirmed *I. pseudacorus* localities were mapped using ArcMap 10.8.1 (ESRI, 2020). To investigate the potential effects of climate on the distribution and abundance of *I. pseudacorus* populations, mean annual temperature and mean annual precipitation overlays were obtained from gis.elsenburg.com (SA Atlas of Climatology and Agrohydrology, R.E. Schulze, 2009). The climate overlays are presented separately, with the distribution and abundance of South African *I. pseudacorus* populations displayed therewith.

2.2.1.3 Statistical Analyses

Using the field data collected in spring and winter of 2019 and 2020 respectively, the mean number of both flowers and capsules per reproductive stem was determined for each site. To investigate whether site had an effect on the number of flowers or capsules produced, separate ANOVAs were performed, comparing i) the mean number of flowers, and ii) the mean number of capsules produced per stem at each of the different sites. The data were found to be normally distributed, and Levene's test confirmed the homogeneity of variance. However, with regard to the flowering data, the pattern observed when plotting the residuals suggested a transformation may be required, and so the data underwent a square root transformation to better conform to the assumptions of the test. Thereafter, sites were grouped by 'climate zone' (adapted from the University of Cape Town's CIP database), and linear mixed effects models were performed with adjustments made for unbalanced data. All analyses were performed using R software (R Core Team, 2020).

2.2.2 Germinative Power

In addition to the capsules collected during the second nationwide survey, dehisced seed capsules were collected at 19 selected sites to assess their germinative powers. The sites selected for this study were chosen based on the maturity of the capsules. Sites at which capsules were immature (not yet dehisced) were excluded, as was the case for sites at which all seeds had already been released from the capsules. At the selected sites, the seeds collected were dried with tissue paper and stored in paper envelopes to transport back to Rhodes University for further laboratory use. Upon arrival, the dried seeds were placed in darkened amber jars filled with distilled water, which were kept at 5 ˚C for a period of three months (April – June 2020), a slight adaptation of a procedure found necessary by Coops and Van der Velde (1995).

Seeds were germinated in petri dishes (8.5 cm diam.) with a sheet of filter paper placed in each dish as a substrate for the seeds. Three petri dishes were used for each of the 19 sites assessed, with 10 seeds in each dish, totalling 30 seeds per site and 570 seeds in total. The dishes were sealed with lids and placed at random on plastic trays sealed with cling wrap to reduce evaporation. The trays were placed in a controlled environment chamber provided by Rhodes

University's Department of Zoology and Entomology, where the seeds were exposed to temperatures between 15-25 ˚C and a photoperiod of 12 hours light: 12 hours dark, a slight adaptation of the methodology followed by Coops and Van der Velde (1995). The seeds were sprayed daily with distilled water and the number of germinated seeds (those in which the radicle had protruded from the testa) was recorded every day for a period of 6 weeks.

2.2.3 Competition

2.2.3.1 Experimental Design

Following an adaptation of Mopper et al. (2016), a common garden competition study was conducted in a greenhouse provided by Rhodes University's Department of Botany. With the exclusion of salinity assessed by Mopper et al. (2016), a full-factorial design with replication assessed the competitive interactions between *I. pseudacorus* and *T. capensis.* This design included the effects of species (*I. pseudacorus* and *T. capensis*), and competition (no competition, intraspecific competition, and interspecific competition), with each treatment replicated six times.

Full *I. pseudacorus* and *T. capensis* plants with intact rhizomes were harvested from Little Timbers, Hogsback, Eastern Cape (-33.16063; 27.43869) in March 2020, and brought back to Rhodes University to be cultivated under similar conditions prior to their use in the study. The cultivations were maintained for five months, after which plants were harvested and cleaned of sediment. Rhizome segments of similar sizes were cut from each plant, and their roots and shoots were trimmed for standardisation. The rhizome segments were weighed and planted in their respective pots at a depth of approximately 2 cm. Following the methodology of Mopper et al. (2016), the number of rhizomes planted per pot was dependent on the competition treatment, with the pots assigned to the "no competition" treatment containing a single rhizome of either *I. pseudacorus* or its competitor, "intraspecific competition" comprising four rhizomes of the same species, and "interspecific competition" comprising two rhizomes from each of *I. pseudacorus* and *T. capensis*, totalling four rhizomes per pot. Both the initial and final biomass data were recorded per species per pot (i.e. all four rhizomes weighed together in the case of the intraspecific competition treatment), and averaged to determine the mean biomass gained by a single rhizome in each treatment.

For each replicate, a round plastic pot (25 cm diam. x 20 cm) was filled with 6 kg of pond sediment obtained from Jameson Dam, Makhanda (-33.3176511; 26.440962). The pots were placed at random in a pool of water to ensure that the sediment was kept saturated throughout the study, as was found necessary in previous attempts.

The experiment was concluded after a growth period of six months, after which the plants were harvested, cleaned of sediment, and air-dried in the sun for two hours. The plants were separated into above- and below-ground biomass, with above-ground biomass comprising the leaves and shoots, and below-ground biomass comprising the roots and rhizomes. The above- and below-ground biomass (wet) was recorded, and the separated plant material was placed into individual brown paper bags, after which it was oven-dried (65 °C for 72 h). The dry biomass of the plant material was recorded per species per treatment, and was standardised to represent the growth of a single plant.

2.2.3.2 Statistical Analyses

All statistical analyses were conducted using R ver. 4.0.3 (R Core Team, 2020).

i) Relative Growth Rate (RGR)

The relative growth rate (RGR) of both *I. pseudacorus* and *T. capensis* were calculated for each replicate in each competition treatment, and were compared between species and across competition treatments. Relative growth rate (RGR) can be defined as the increase in biomass over time, accounting for initial biomass, and is calculated as follows:

$$RGR = (\ln W_2 - \ln W_1)/(t_2 - t_1),$$

where W_1 and W_2 are plant dry weight at times t_1 and t_2 respectively.

After confirming that the data conformed to the relevant assumptions, a two-way ANOVA was performed to investigate the potential effects of plant species and competition (separately and interactively) on RGR. This was followed by Tukey’s HSD post hoc test to determine where significant differences occurred.

ii) Root/Shoot Ratio (RSR)

The allocation of accumulated biomass was determined and compared for each species in each competition treatment using the root/shoot ratio (RSR). The RSR provides information on the relative allocation of resources between above- and below-ground plant tissue, and can be a useful measure to indicate competitive interactions occurring between plants (Qi et al., 2019). As the data conformed to the relevant assumptions, a two-way ANOVA was performed to investigate the effects of plant species and competition (separately and interactively) on RSRs. This was followed by Tukey's HSD post hoc test to determine where significant difference occurred.

iii) Relative Intensity Index (RII)

For each competition treatment, the mean total, above- and below-ground biomass accumulation of *I. pseudacorus* and *T. capensis* were compared. Using the relative intensity index (RII) (Armas et al., 2004), the competitive (and/or facilitative) interactions between *I. pseudacorus* and *T. capensis* were investigated (Balestri et al., 2021). The RII values range from -1 to 1, with negative values suggesting competitive interactions have occurred, while positive values indicate the presence of facilitative interactions. The RII was calculated separately for each of the competing species, and included the effects of both intra- and interspecific competition. The RII for intraspecific competition was calculated as:

$$RII = (B_{high} - B_{low}) / (B_{high} + B_{low}),$$

where B_{high} is the mean biomass of the plants grown at high density, and B_{low} is the biomass of the single plant grown alone. In the presence of a competing species, the intensity of the interspecific interactions is calculated as:

$$RII = (B_{with\ competitor} - B_{without\ competitor}) / (B_{with\ competitor} + B_{without\ competitor}),$$

where $B_{with\ competitor}$ is the mean biomass of either *I. pseudacorus* or *T. capensis* when grown in combination with one another, and $B_{without\ competitor}$ represents the mean biomass of either species when grown without the presence of the other, but at the same planting density. Plants that did not survive the duration of the experiment were included in the RII calculations, and were recorded as having a biomass of zero.

One-sample mean *t*-tests were used to test each RII value for significant departures from zero, i.e. to determine whether significant (competitive or facilitative) interaction occurred (Balestri et al., 2021). Prior to conducting the one-sample mean *t*-tests, data were tested for normality. In one case in which normality assumptions were violated, a nonparametric one-sample Wilcoxon signed-rank test was used in place of the *t*-test.

2.3. Results

2.3.1 Distribution and Density

Iris pseudacorus infestations have been recorded in eight of South Africa's nine provinces, with the exception of the arid Northern Cape (Figure 2.1). At present, there are 50 confirmed records of *I. pseudacorus* infestations in South Africa, excluding any reported infestations which my colleagues or I have not physically ground-truthed (Table 2.1). Many more than 50 *I. pseudacorus* infestations have been recorded (Figure 2.2), but unfortunately not all sites were accessible, and many records provided by citizen scientists were not accompanied by photographs, so these sites have not been included in this study. For example, *I. pseudacorus* was not present at 13 sites where its presence was recorded by citizen scientists, who may have mistaken the yellow *Canna indica* L. (Cannaceae) for *I. pseudacorus*, as this species was often present at the coordinates provided.

Table 2.1: GPS coordinates (decimal degrees) of 50 confirmed South African *Iris pseudacorus* populations, including the season in which sampling occurred (S = Spring, flowering; W = Winter, seeding, blank = too small to sample), and whether tissue samples were collected for genetic analysis (*)

Prov.	**Site Name**	**Abbr.**	**Latitude**	**Longitude**	**Sampling**
GAU	Vanderbijl Park, roadside	VBP	-26.66722	27.84056	S
GAU	Vanderbijl park, towards Parys	VB	-26.71194	27.80194	S, W *
GAU	PTA, Equestria, park	EQPTA	-25.75083	28.30861	S
GAU	PTA, Equestria, houses	PTA	-25.76139	28.3275	S
GAU	JHB, Mushroom Park, Sandton	SMP	-26.105	28.06361	S, W
GAU	JHB, Witkoppen Park	WPJHB	-26.0105	28.00705	S
GAU	JHB, Witkoppen Road	-	-26.02944	27.975	
GAU	JHB, Witkoppen Road, stream	-	-26.03028	27.97305	
GAU	JHB Botanical Gardens, small pond	-	-26.16167	28.00005	
GAU	JHB Botanical Gardens, Emmarentia	EG	-26.15333	28.99972	S, W *
GAU	JHB Walkhaven Dog Park	WH	-25.99528	27.86528	S, W *
GAU	JHB God First Church	GFC	-26.9	27.97194	S
GAU	JHB St Stithians School	SSS	-26.5013	28.1583	S
GAU	JHB Glenhazel Primary School	GH	-26.134429	28.102318	S, W *
GAU	JHB, Emma Park, Linden	EP	-26.13656	27.983255	S *
GAU	JHB, Zoo Lake	ZL	-26.92332	28.14461	S, W *
GAU	JHB, Kya Sands	KSAI	-26.01412	27.571979	S
NW	Hart~~e~~beespoort Dam	-	-25.76222	27.81306	
NW	Groot Marico, Riverstill Guest Farm	GM	-25.637687	26.43137	W *
MPU	Dullstroom, Dunkeld Country Estate	DK	-25.235907	30.45457	S, W *
MPU	Dullstroom, Mavungana Flyfishing	-	-25.424256	30.100415	

MPU	Dullstroom, Walkersons Estate	WW	-25.2281	30.11414	S, W *
LIM	Bela-Bela, Klein Kariba Resort	KK	-24.83333	28.33333	S, W *
LIM	Haenertsburg, Lekwar Restaurant	LKW	-23.93972	29.94083	S, W *
LIM	Magoebaskloof, in horse paddock	MGK	-23.87889	29.90778	S, W *
KZN	Curry’s Post, Heavenly Haven	LR	-29.43364	30.15723	S *
KZN	Terbodore Coffee Shop	TBD	-29.35862	30.1403	S
KZN	Howick, Merrivale	HW	-29.51306	30.23111	
KZN	Merrivale, Celtiskloof	MVC	-29.52168	30.23707	S
KZN	Durban Botanical Gardens	DB	-29.84782	31.00724	S *
KZN	Hillcrest, Assegai River	HC	-29.84606	31.00564	S
KZN	PMB Botanical Gardens	PMB	-29.60782	30.34567	S *
KZN	PMB, Ascott Bush Lodge	ABL	-29.60249	30.40944	S, W *
KZN	Rosette Village Dam	RD	-29.3051	29.974792	S *
KZN	PMB, Wylie Park	WP	-29.588864	30.350881	S
WC	CT, Diepriver	-	-34.0147	18.4561	
WC	CT, Constantia stream	CCT	-34.0336	18.4414	S, W
WC	CT, Contstantia Park	CP	-34.0228	18.4324	S, W *
WC	CT, Hoerskool Jan van Riebiek	HJVR	-33.9342	18.4089	S, W *
WC	CT, Wyneberg Park	WBP	-34.0017	18.4536	*
WC	CT, Newlands, Vineyard Hotel	NVH	-33.979145	18.458885	
WC	CT, Noordhoek Commons	NH	-34.097591	18.375496	S, W *
WC	Farm Vermaaklikheid	VMK	-33.31	21.03	S
EC	GHT, Belmont Valley Farm	-	-33.30635	26.52525	
EC	GHT, Belmont Valley Golf Club	BVG	-33.3228	26.61375	
EC	EL, Ronnie's Motors	RM	-32.94878	27.92327	
EC	EL, Lavender Blue	LB	-32.94268	27.93356	
EC	EL, Beacon Bay Virgin Active	-	-32.952556	27.930639	
EC	Hogsback, Little Timbers	LT	-33.16063	27.43869	S, W
FS	Bloemfontein, Shell Ultra City	-	-28.888772	26.251581	

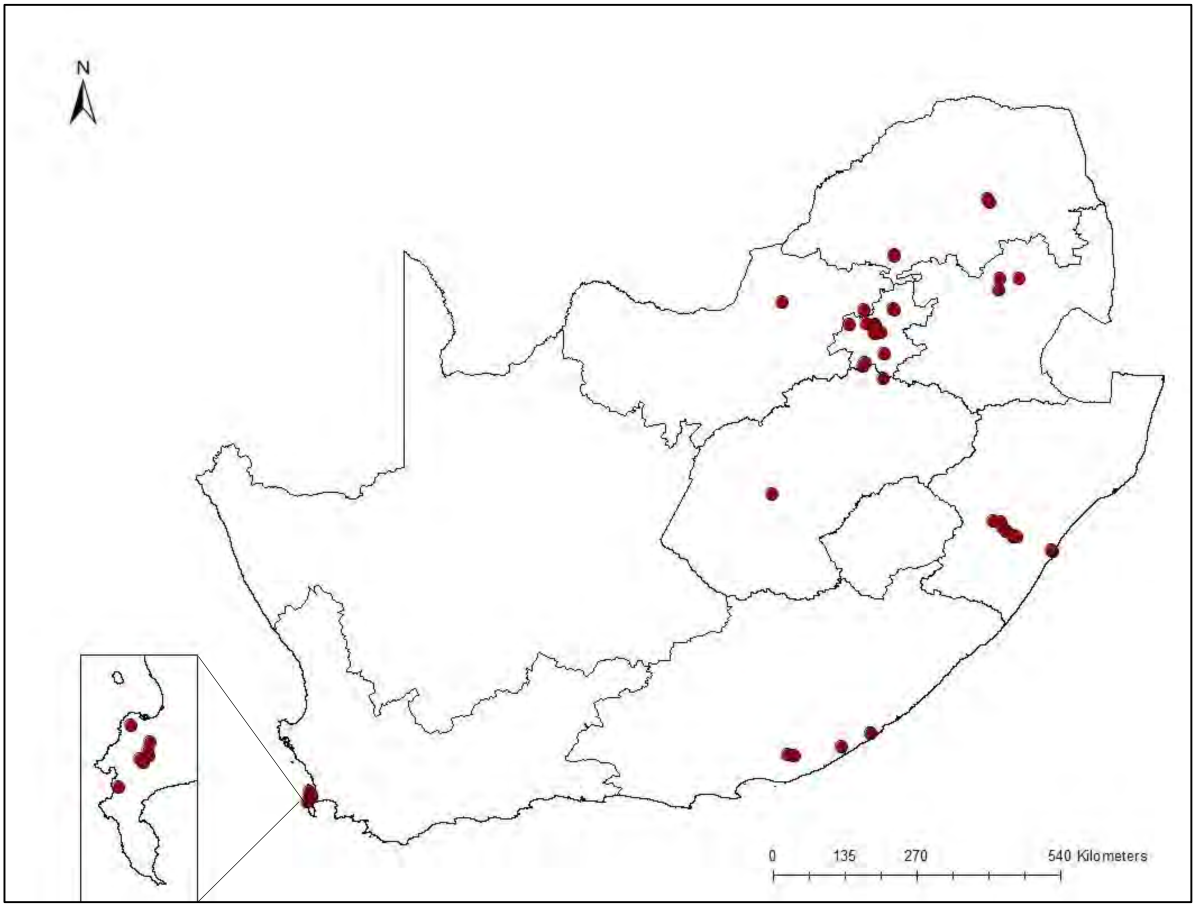


Figure 2.1: Distribution of confirmed *Iris pseudacorus* populations in South Africa, sampled between October-November 2019 and February-March 2020

By 2015, Jaca and Mkhize (2015) reported 24 populations of *I. pseudacorus* in South Africa (Figure 2.2). A substantial increase in South African *I. pseudacorus* records was observed in 2018, with the number of records increasing from 24 to 63 (Figure 2.2). A further 35 records were obtained in 2019, increasing the total number of records to 98 (Figure 2.2). An additional 7 records were obtained in 2020, and an additional 12 were obtained by October 2021, totalling 117 reports of *I. pseudacorus* populations in South Africa (Figure 2.2).

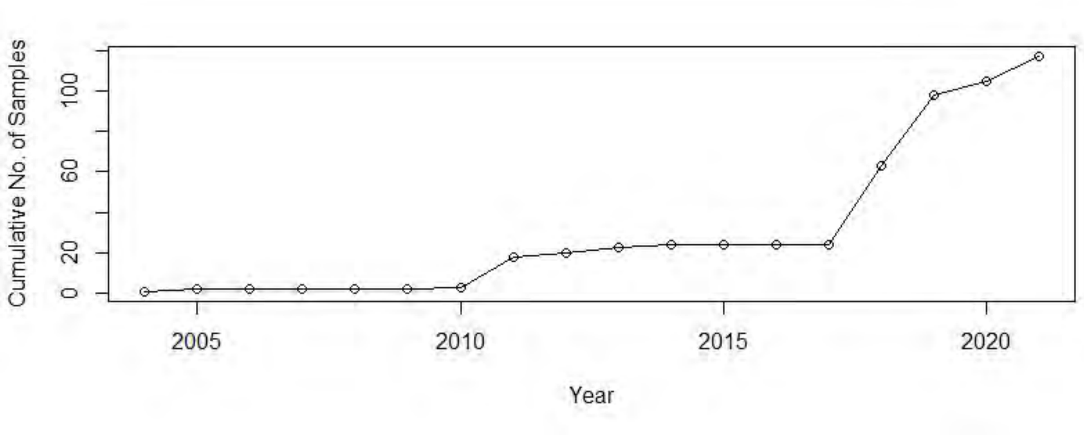
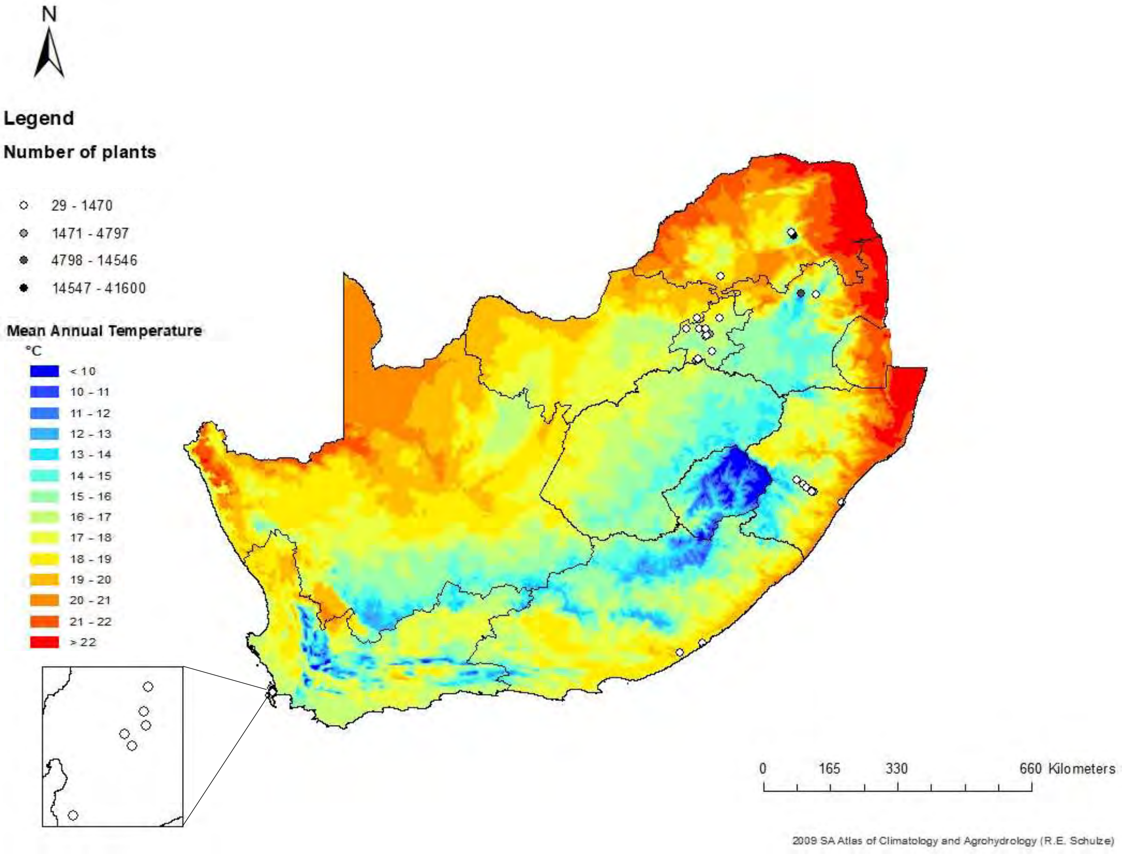


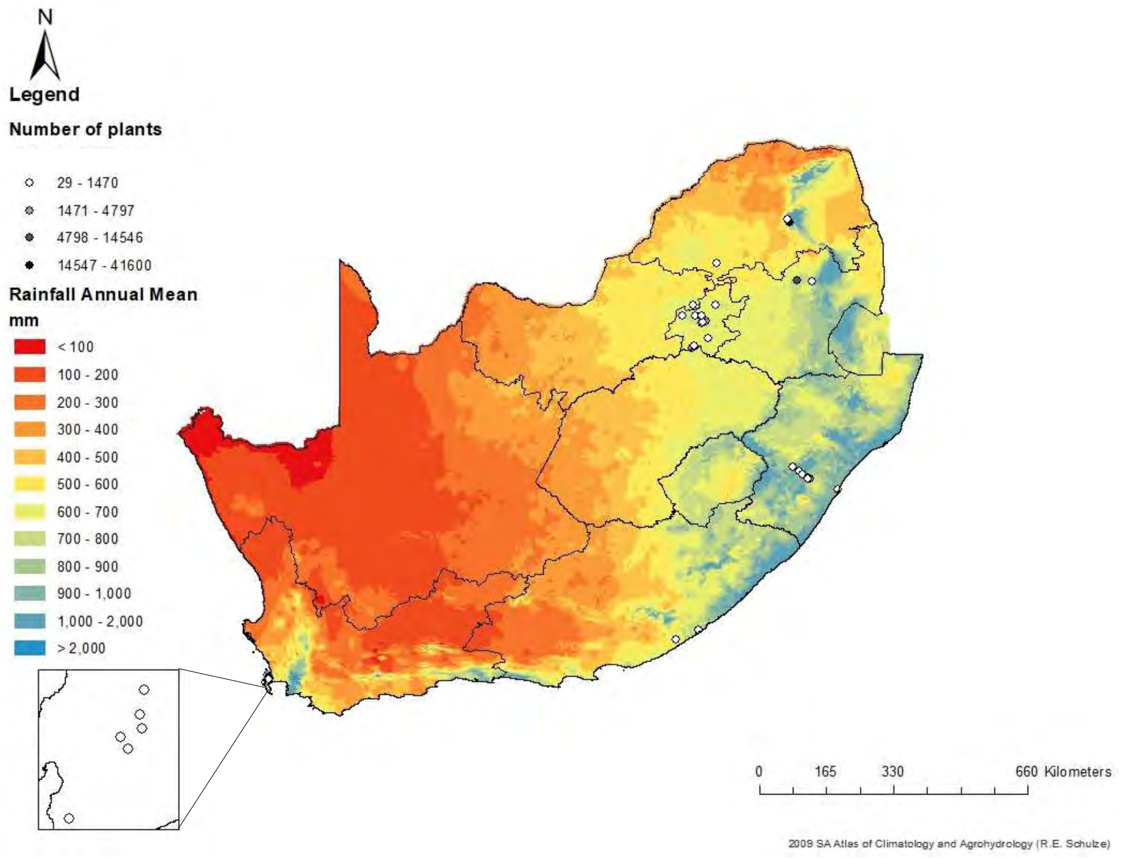
Figure 2.2: Cumulative records of South African *I. pseudacorus* populations recorded from 2004 to 2021

Although fewer in number, South African *I. pseudacorus* infestations were most severe (in terms of abundance) in the Limpopo and Mpumalanga provinces, where both the mean annual temperature and precipitation are notably high (Figure 2.3). The number of infestations recorded was highest in the country's major cities (Johannesburg, Pretoria, Cape Town, and Durban) and adjoining areas (e.g. Pietermaritzburg), and was substantially lower in the Eastern Cape, the Free State, Limpopo and Mpumalanga (Figure 2.1, Figure 2.3). While there were more records of *I. pseudacorus* populations in and around major cities, these infestations were not as severe as those in Limpopo and Mpumalanga (Figure 2.3).



2009 SA Atlas of Climatology and Agrohydrology (R.E. Schulze)

a)



2009 SA Atlas of Climatology and Agrohydrology (R.E. Schulze)

b)

Figure 2.3: Distribution and abundance of *Iris pseudacorus* populations in South Africa, sampled between October – November 2019, with a) mean annual temperature overlay; and b) mean annual precipitation overlay. Relative abundance is illustrated by the shading of the circles.

2.3.2 Reproductive Potential

South African *I. pseudacorus* populations produced an average (± S.E.) of 2.570 (± 0.191) stems per m^2, with 7.434 (± 0.363) flowers and 3.720 (± 0.358) capsules per stem (Table 2.2). Each capsule produced an average of 42.506 (± 1.853) seeds, with 83.333 % (± 0.244) seeds having the ability to germinate after a period of 6 weeks (Table 2.2). This translates to 406.370 seeds per m^2, with approximately 338.632 seeds having the ability to germinate.

Table 2.2: Mean sexual reproductive output of *Iris pseudacorus* populations in South Africa, sampled between October-November 2019 and February-March 2020

	No. Reproductive Stems per m²	No. Flowers per Stem	No. Capsules per Stem	No. Seeds per Capsule	Germinable Seed (%)
Mean	2.570	7.434	3.720	42.506	83.333
Standard Error	0.191	0.363	0.358	1.853	0.244

South African *I. pseudacorus* seeds showed high germinability, with ~ 83 % of seed germinating after a period of six weeks (Table 2.2, Figure 2.4). An exponential increase in germinated seed was observed, with 50 % of the seed having germinated by day 16, and the remaining ~ 33 % of seed germinating over the next ~ 3.7 weeks (Figure 2.4).

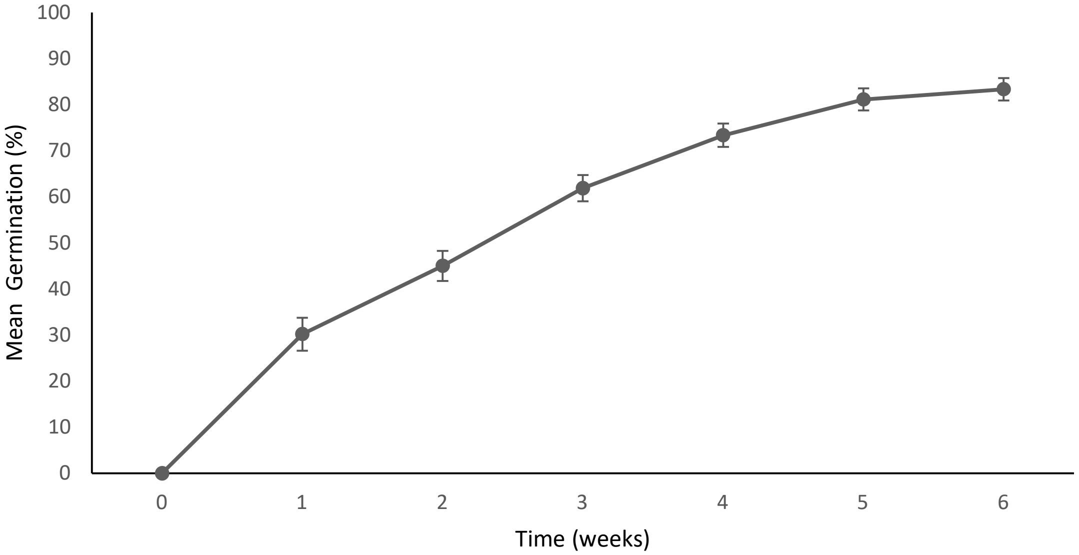


Figure 2.4: Mean germination rate (± S.E., n = 54) of South African *Iris pseudacorus* seeds over a period of six weeks

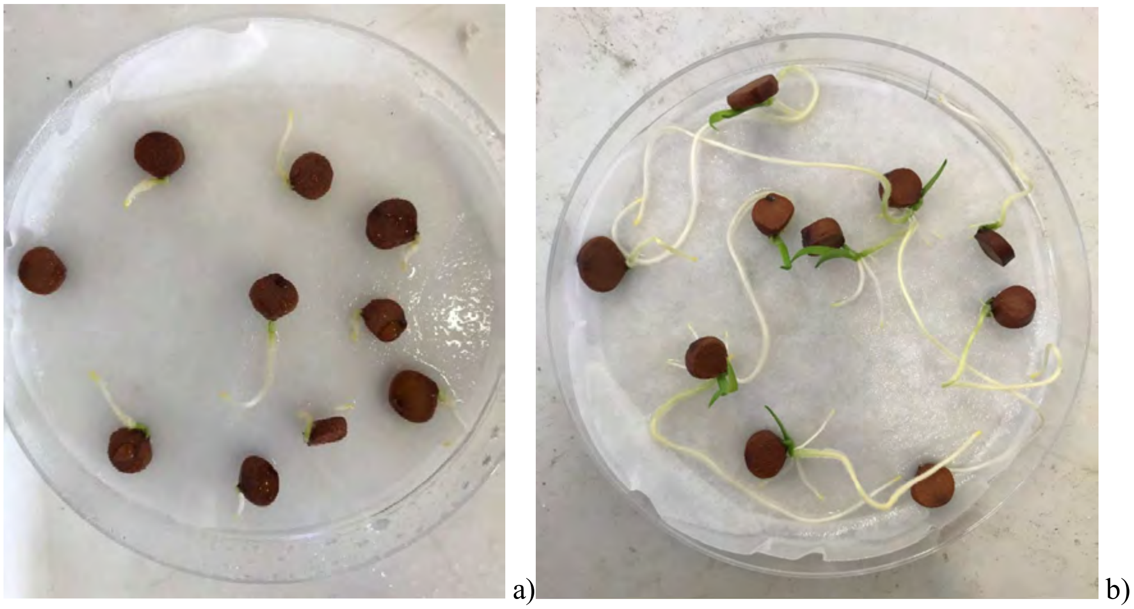


Figure 2.5: Germinating *Iris pseudacorus* seeds after a period of a) two, and b) six weeks in a controlled environment (photos by E Sandenbergh, 2020)

The mean (±S.E.) number of flowers produced per stem across the 34 sites sampled ranged from 0.666 (±1.155) to 12.5 (±0.707) flowers per stem (Figure 2.6). The mean (± S.E.) number of seed capsules produced per stem was less variable, ranging from 0.8333 (± 0.764) to 5.111 (± 1.388) seed capsules per stem (Figure 2.7). The number of flowers and capsules produced did not differ significantly between sites (F = 1.546, *P* = 0.0683; F = 1.349, *P* = 0.225) respectively), suggesting site has no effect on sexual reproductive output (Figure 2.6, Figure 2.7). However, with regard to 'climate zone', the Western Cape 'climate zone' produced significantly fewer flowers than the other climate zones (t = -2.178, *P* = 0.0399), but no climate effect was observed for capsule production (Figure 2.6, Figure 2.7).

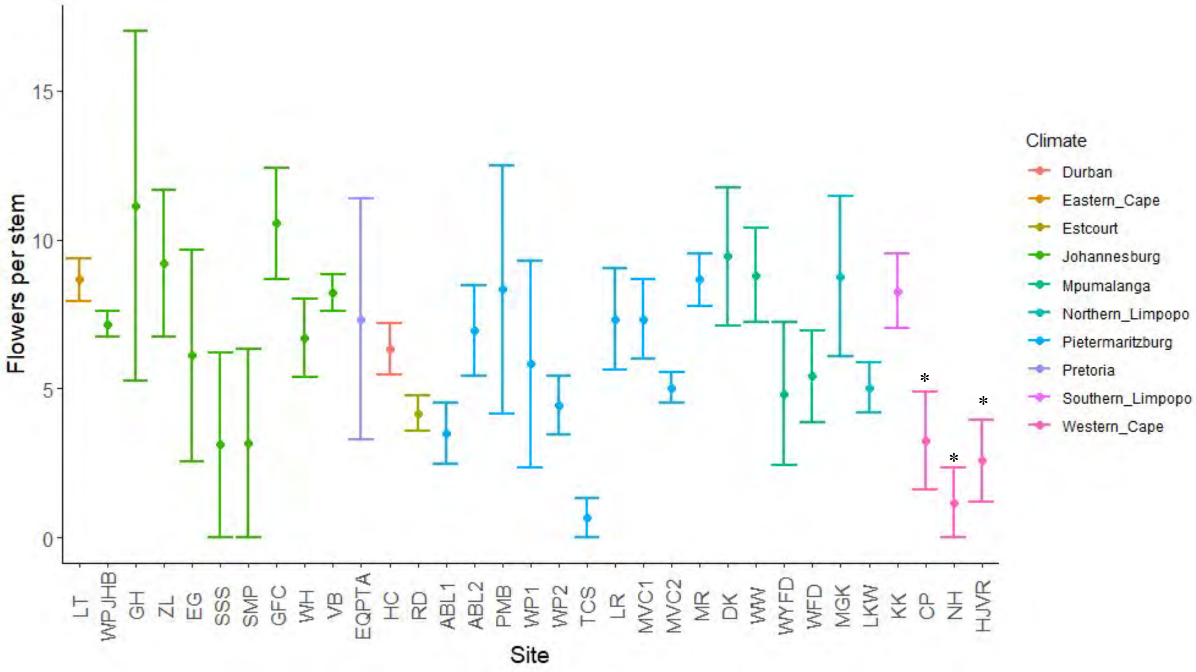


Figure 2.6 Mean (± S.E.) number of flowers produced per stem by various South African *Iris pseudacorus* populations, sampled between October-November 2019. Asterix indicates significant difference in flower production ($P < 0.05$). Sites at which multiple invasions occurred and sampling occurred in two separate areas are numbered, eg. “ABL1” and “ABL2”. Site abbreviations defined in Table 2.1 above.

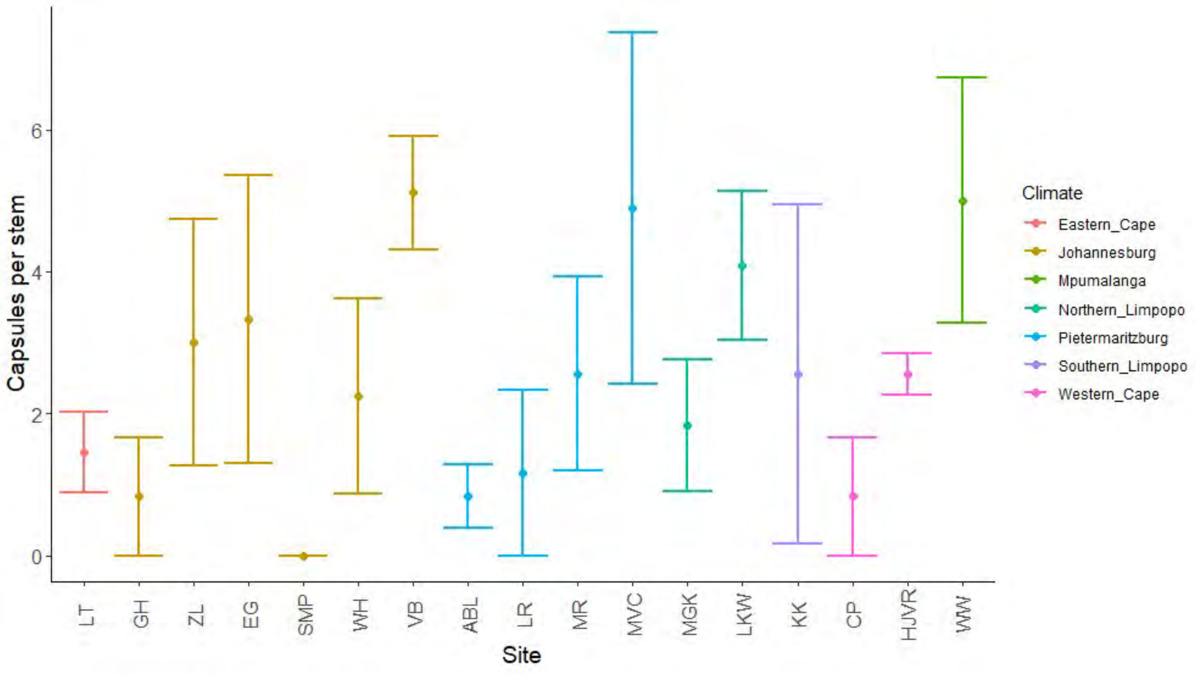
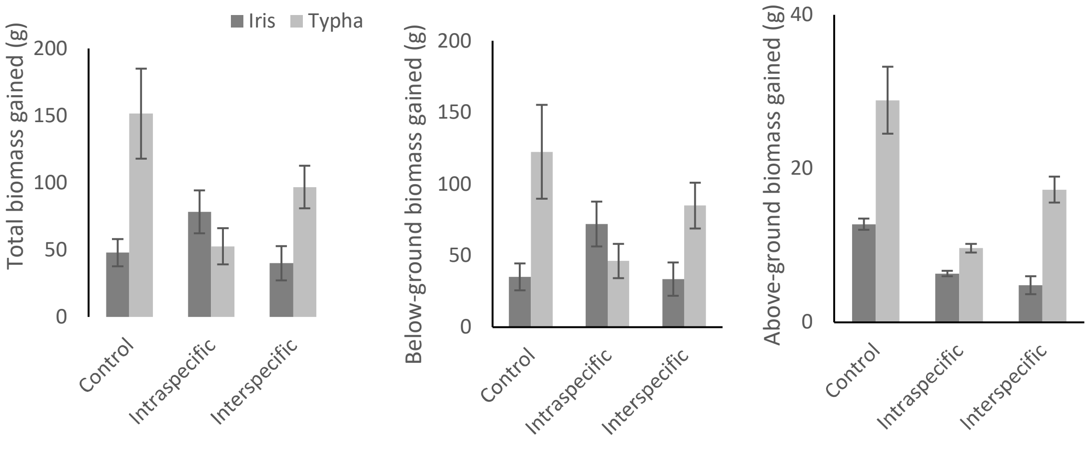


Figure 2.7: Mean (± S.E.) number of capsules produced per stem by various South African *Iris pseudacorus* populations, sampled between February-March 2020. Site abbreviations defined in Table 2.1 above.

2.3.3 Competition

Typha capensis accumulated more biomass both above- and below-ground than was the case for *I. pseudacorus* in both the control and interspecific competition treatments, as well as accumulating more above-ground biomass in the intraspecific competition treatment (Figure 2.8). For both species and for all treatments, biomass accumulation was greater below- than above-ground, thus contributing more to the total biomass accumulated. Relative to the control, total and below-ground biomass of *I. pseudacorus* increased substantially in the presence of other *I. pseudacorus* individuals (Figure 2.8). Conversely, in the presence of *T. capensis*, the total and below-ground biomass accumulation of *I. pseudacorus* was slightly diminished (Figure 2.8). However, with regard to above-ground biomass accumulation, interspecific competition exerted by *T. capensis* appeared to have a greater impact on *I. pseudacorus* than was the case for intraspecific competition (Figure 2.8). Regarding *T. capensis*, both inter- and intra-specific competition reduced total, above- and below-ground biomass accumulation, with intraspecific competition having a greater effect (Figure 2.8).



a) b) c)

Figure 2.8: Mean (± S.E.) a) total, b) below- and c) above-ground biomass of *Iris pseudacorus* and *Typha capensis* grown under three competition treatments

The relative growth rate (RGR) of *T. capensis* was consistently higher than that of *I. pseudacorus* under all three competition treatments (Figure 2.9). While there was no effect of competition treatment on RGR (F = 0.137; $P = 0.873$), plant species had a significant effect on RGR (F = 8.538, $P = 0.0065$), with *T. capensis* significantly outperforming *I. pseudacorus* in the interspecific treatment (Tukey’s HSD, $P = 0.027$) (Figure 2.9).

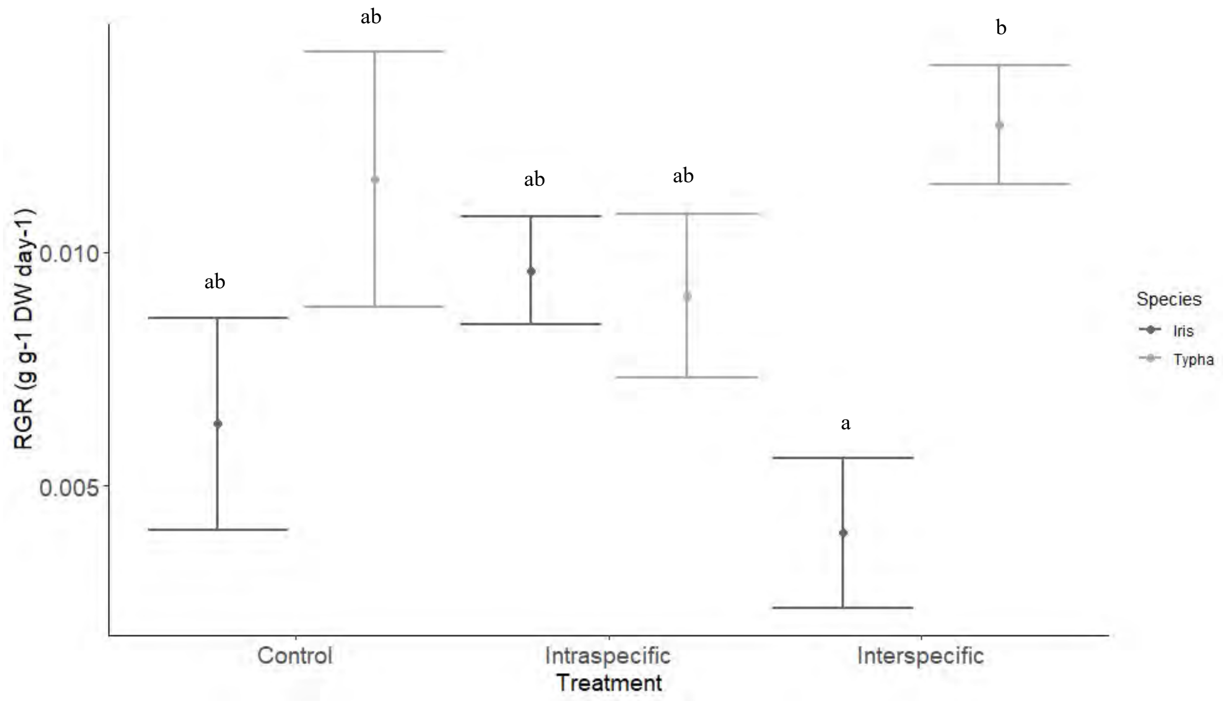


Figure 2.9: Mean (± S.E.) relative growth rates (RGRs) of *Iris pseudacorus* and *Typha capensis* under three competition treatments. Different letters denote significant differences between species within treatments ($P < 0.05$).

Plant species did not have a significant effect on the root/shoot ratio (RSR) (F = 1.874, P = 0.181), but RSR was significantly affected by competition treatment (F = 7.649, P = 0.002), as well as by the interactive effect of plant species and competition treatment (F = 4.092, P = 0.026) (Figure 2.10). The RSR of *I. pseudacorus* in the intraspecific competition treatment was significantly greater than that of both itself (P = 0.0006) and *T. capensis* (P = 0.008) in the control treatment (Figure 2.10).

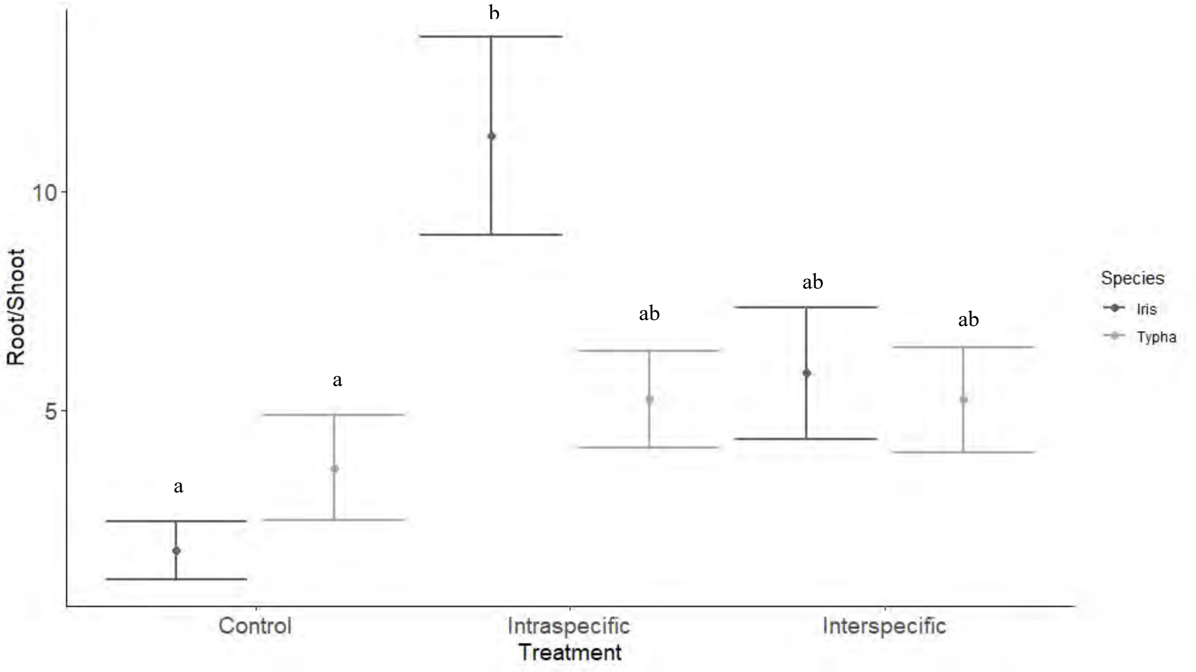
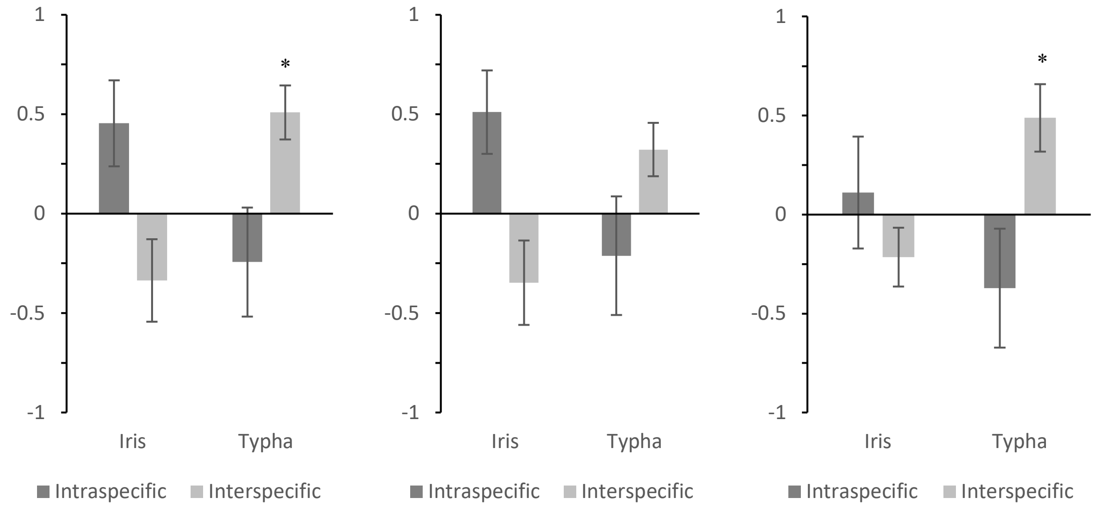


Figure 2.10: Mean (± S.E.) root/shoot ratios (RSRs) of *Iris pseudacorus* and *Typha capensis* under three competition treatments. Different letters denote significant differences in the interactive effect of plant species and competition treatment ($P < 0.05$).

No significant departures from zero were observed in the RII values for *I. pseudacorus*, indicating a lack of significant interactive effects, either competitive or facilitative (Figure 2.11). A significant departure from zero was observed in the RII values for *T. capensis* for both total ($t = 2.617$, $P = 0.047$) and above-ground RII ($t = 2.869$, $P = 0.035$) in the interspecific treatment, both of which indicate a facilitative effect from competition with *I. pseudacorus* (Figure 2.11).



a) Total b) Below-ground c) Above-ground

Figure 2.11: a) Total, b) below- and c) above-ground relative intensity indices (RIIs) of *Iris pseudacorus* and *Typha capensis* under three competition treatments. Asterix indicates significant departure from zero ($P < 0.05$).

2.4 Discussion

2.4.1 Distribution and Abundance

The distribution of *Iris pseudacorus* in South Africa is broad, with infestations reported in all but one of the country's provinces. As of October 2021, 50 *I. pseudacorus* infestations have been confirmed, doubling the reported 24 populations referred to by Jaca and Mkhize (2015). While only 50 *I. pseudacorus* populations were confirmed through surveys and ground-truthing, just over 100 infestations have been reported, but were inaccessible due to distance, private property, inaccurate GPS coordinates and/or physical barriers.

It is likely that many more than the reported 24 infestations existed in 2015, and that the increasing participation of citizen scientists in South Africa is partly responsible for the two-fold increase in *I. pseudacorus* records. Many of the new infestation reports obtained during and prior to this study were citizen science reports sent in response to a social media campaign initiated by Prof. Julie Coetzee and Ms Samella Ngxande-Koza (CBC) in 2018. Citizen science, or the involvement of the public in scientific research, has proven highly valuable to many fields, including that of invasion ecology and invasive species management (Davis et al., 2018; Gervazoni et al., 2020). Through social media campaigns and the involvement of citizen scientist volunteers, a large amount of distribution data can be obtained at a low cost while actively engaging the public in biodiversity conservation, thus contributing to environmental education and awareness (Gervazoni et al., 2020).

While the use of citizen science platforms can be of great value in invasive species management, the data generated by non-specialists must be used with caution. As those unfamiliar with certain species may provide incorrect identifications, and those unfamiliar with certain technologies may provide inaccurate coordinates, the data generated by non-specialists should be confirmed, be it through ground-truthing or the provision of clear photographs. For example, in this study, citizen scientists often mistook the invasive *Canna indica* L. (Cannaceae) for *I. pseudacorus*, as both have showy yellow flowers which could easily be confused when viewed at a distance or at a roadside site. As such, a conservative approach was taken, in which infestation reports which were not accompanied by photographs, and which could not be accessed, were not included.

New populations of *I. pseudacorus* are being established artificially by horticulturalists and gardening enthusiasts who are spreading the weed without knowledge of its invasive status. Due to its attractive flowers, its durability, and its ability to spread rapidly, *I. pseudacorus* is a popular choice of pond plant in many residential and recreational landscapes, and can easily be cultivated from either rhizome fragments or seed. Unfortunately, despite being a category 1a invasive plant (NEMBA, 2004), it has been reported that various nurseries in South Africa have been selling the weed, and gardening enthusiasts have shared plants amongst themselves, contributing to its spread. Furthermore, the species was promoted for use in the trout industry and on golf courses, likely due to its ability to remove pollutants from water bodies, further contributing to the spread of the invader. For example, the *I. pseudacorus* populations in Dullstroom, Mpumalanga, exist as a result of the species' use in the trout industry (pers. obs.).

New *I. pseudacorus* populations may establish from cultivated populations through natural means of dispersion, including the fragmentation of rhizomes and the dispersal of seed. 'Escaping' cultivation has been reported as a main pathway of establishment and invasion of *I. pseudacorus*, with both rhizomes and seeds well adapted to hydrochory, or the dispersal of seeds via water systems (Morgan et al., 2020; Minuti et al., 2021). As discussed further in Chapter 3 of this thesis, the high levels of genetic diversity between and within populations of *I. pseudacorus* suggest that sexual reproduction is the primary method of reproduction employed by the species, and so, with regard to distribution, the effects of seed dispersal would likely outweigh those of rhizome fragmentation.

The majority of South African *I. pseudacorus* infestation records come from the country's major cities, including Johannesburg (13 confirmed sites) and Cape Town (7 confirmed sites). A large number (10 confirmed sites) of records also come from Kwa-Zulu Natal, with most of the province's infestations occurring in and around the Pietermaritzburg/Howick area. This finding was not surprising, as Gauteng, Kwa-Zulu Natal and the Western Cape have the highest human population estimates, accounting for 25.8 %, 19.2 % and 11.6 % of the country's population respectively, and 56.6 % of the country's population cumulatively (ststssa.gov.za, 2019), with the country's most highly populated cities being Cape Town, Durban, and Johannesburg. As *I. pseudacorus* is largely spread through the horticultural industry, the dense human populations in these areas likely account for the high number of *I. pseudacorus* infestations due to a greater frequency of cultivation and thus a greater likelihood of 'escape' (McLean et al., 2017). Furthermore, positive relationships between the species richness of alien plants and the density of human populations have been reported, with the horticultural industry identified as a major pathway for invasions in urban areas (Hodkinson and Thompson, 1997; Reichard and White, 2001).

While South Africa's major cities contain the highest numbers of *I. pseudacorus* populations, the infestations in these regions are not particularly severe. The severity of infestations, measured as a function of density and area cover (i.e. abundance), appears to be linked to both temperature and precipitation, although there is insufficient data to draw any firm conclusions, as *I. pseudacorus* still appears to be in the "lag" phase of its invasion in this country (Blackburn et al., 2011). The distribution and abundance data, overlaid with both the mean annual temperature and mean annual precipitation data suggest that *I. pseudacorus* occurs predominantly in subtropical regions with high rainfall.

The climate data alone, however, are insufficient to explain the distribution pattern of *I. pseudacorus* in South Africa, as many populations are those which have been cultivated or have recently 'escaped' cultivation. While it is certain that *I. pseudacorus* cannot survive past certain climatic thresholds, such conclusions cannot be drawn from the distribution data collected in this study, as it is likely that anthropogenic factors strongly influence the current distribution of *I. pseudacorus* in South Africa.

As *I. pseudacorus* is still in the 'lag' phase of its invasion in South Africa, it may be useful to subset the confirmed localities, excluding 'artificial' sites, and focusing only on those which could be classified as invasions. With a reduced sampling size, more comprehensive surveys could be conducted, incorporating abiotic factors such as soil and water nutrient concentrations, dissolved oxygen, pH, and conductivity, which influence the distribution, abundance, and sexual reproductive output of *I. pseudacorus* populations, and provide more insight into the species' invasion ecology. While these data may be insufficient to draw any firm conclusions, the distribution pattern observed agrees with the findings of Minuti et al. (2022), in that temperature and precipitation are likely amongst the most influential climatic factors in determining *I. pseudacorus* distributions.

Although the current distribution of South African *I. pseudacorus* populations may be influenced more by anthropogenic than climatic factors, the abundance data suggest that the severity of invasion is closely associated with climate. While the country's major cities and highly populated regions contain the majority of invasion records, the severity of invasion in these regions is not particularly high. The Limpopo and Mpumalanga provinces, on the other hand, contain fewer records of *I. pseudacorus* populations, but the severity of invasion in these provinces is relatively high. As *I. pseudacorus* prefers warm, wet climates (Minuti et al., 2022), it is understandable that the species thrives in these regions.

2.4.2 Reproductive Potential

The success of an invasive species depends largely on its ability to acquire and utilise resources in an invaded landscape, while the ways in which such resources are utilised has substantial effects on the invasive potential of a species. The invasive potential of a species depends largely on its reproductive potential, or its ability to reproduce and propagate effectively in a novel environment, as well as its ability for propagule dispersal (Barrett et al., 2008; Gaskin et al., 2016). The reproductive potential of an invasive species is often enhanced when introduced outside of its native range (Crawley, 1987; Blossey and Nötzold, 1995). This could be due to a variety of reasons, including the potential reallocation of resources in the absence of natural enemies (Blossey and Nötzold, 1995; Keane and Crawley, 2002; Joshi and Vrieling, 2005).

South African *I. pseudacorus* populations were found to have high sexual reproductive outputs, with a mean (± S.E) of 42.506 (± 1.853) seeds produced per seed capsule, 3.720 (± 0.358) capsules and 7.434 (± 0.363) flowers produced per reproductive stem, and 2.570 (± 0.191) reproductive stems per m^2 (Table 2.2). The number of seeds produced per capsule varied greatly, which may have been due to collecting the capsules at different stages of maturity. The sample size for this measure was considerably smaller than that of the others as capsules were not mature throughout all sites, and so were not included.

Few studies have been published on the sexual reproductive output of *I. pseudacorus* in its native range, with very limited data available. Native *I. pseudacorus* individuals have been reported to produce 5-6 seed capsules per plant (n = 8), with the mean number of seeds per seed capsule being reported as 32 (± 1.55, S.E., n = 45) (Sutherland, 1990) and the mean number of seeds per reproductive stem reported as 47 (± 15, S.D.) (Coops and Van der Velde, 1995).

With 42.506 (± 1.853) seeds produced per seed capsule, and 3.720 (± 0.358) capsules produced per reproductive stem, the sexual reproductive output of *I. pseudacorus* populations in South Africa is high. *Iris pseudacorus* produces a vast number (~ 338.632) of germinable seeds per m^2. This has implications for the management of *I. pseudacorus* populations, as the potential seed bank of the species will influence its ability to regrow post-control.

The germination rate reported herein (83.333 %) is far greater than that reported by Coops and Van der Velde (1995) in the Netherlands, who found that only about 25 % of *I. pseudacorus* seeds had the ability to germinate. However, Sutherland (1990) reports an experimental germination rate of 85 %, which decreased with decreasing temperatures, suggesting variability in the germination capabilities of native *I. pseudacorus* populations. While quantitative native range data are limited, these findings suggest that the reproductive output and invasive potential of *I. pseudacorus* may be enhanced outside of its native range, likely as a result of escape from natural enemies. This trend is common amongst invasive plant species, with many studies reporting enhanced seed production in a species' introduced range (Daws et al., 2007). For example, enhanced seed production in the invaded range has been reported for invasive Australian *Acacia* species (Correia et al., 2016), *Cytisus scoparius* (Buckley et al., 2003), and *Chrysanthemoides monolifera* (Scott, 1996).

A comparison of the sexual reproductive output of South African *I. pseudacorus* populations to more recent native range data, as well as to that of other invaded ranges would further support the EICA hypothesis. Seed viability and germination studies have been conducted in the United States, where 99.1 % of seeds collected in Montana were viable (Gaskin et al., 2016) and approximately 96 % of seeds collected in California proved germinable when exposed to freshwater conditions (Gillard et al., 2021). Data from the southern hemisphere is limited, but collaborations are underway with researchers in Argentina and New Zealand, and so a more comprehensive dataset for *I. pseudacorus* invasions in the global south will be available in the near future.

Across the 34 sites surveyed, the mean number of flowers and seed capsules produced per stem did not differ significantly between sites. However, when grouped into 'climate zones' based on proximity to weather stations included in the University of Cape Town's CIP database, the 'WC' 'climate zone' produced significantly fewer flowers than was the case elsewhere, but no climate effect was observed for the production of seed capsules. Climatic factors such as temperature and precipitation are known to affect the phenology and reproductive output of angiosperms, and it is likely that this would hold true for *I. pseudacorus.* However, as *I. pseudacorus* populations in South Africa are largely artificial (i.e. cultivated), the potential anthropogenic factors influencing their behaviour should not be ignored in favour of potential climate effects.

2.4.3 Competition

In both the control treatment and the interspecific competition treatment, the biomass accumulation of *T. capensis* was greater than that of *I. pseudacorus*. In the intraspecific competition treatment, the total and below-ground biomass accumulation of *I. pseudacorus* was greater than that of *T. capensis*. However, the above-ground biomass of *T. capensis* was greater than that of *I. pseudacorus* across all treatments. Under all three competition treatments (control, intraspecific- and interspecific competition), *T. capensis* had a higher RGR than *I. pseudacorus*, with a significantly greater performance in the interspecific competition treatment. The RGR of *T. capensis* was greatest in the control treatment (no competition), and lowest in the intraspecific competition treatment, suggesting the growth of *T. capensis* individuals is sensitive to the competitive interactions exerted by other *T. capensis* individuals, but not particularly sensitive to co-occurring *I. pseudacorus* individuals. However, as discussed below, the presence of *I. pseudacorus* may induce a facilitative response from *T. capensis*. Unlike that of *T. capensis*, the RGR of *I. pseudacorus* was greatest in the intraspecific competition treatment, and lowest in the interspecific competition treatment, suggesting *I. pseudacorus* performs better when grown in combination with other *I. pseudacorus* individuals, but its growth is suppressed when competing against *T. capensis*. As the RGR of a species can influence its competitive ability (Benjamin and Hardwick, 1986), the results obtained suggest *T. capensis* may be a superior competitor than *I. pseudacorus*.

While the RGR of *T. capensis* was consistently higher than that of *I. pseudacorus*, the RSR of *I. pseudacorus* exceeded that of *T. capensis* under the intra- and inter-specific competition treatment, and the values were similar in the control treatment. The RSR provides information regarding the allocation of accumulated resources, and the results obtained indicate that *I.*

pseudacorus allocates a greater proportion of resources to below-ground tissues than is the case for *T. capensis*. *Iris pseudacorus* is known to produce dense rhizomatic mats which exclude co-occurring species (Thomas, 1980; Lamote et al*.,* 2002), and so it was expected that the species would have a high RSR.

The RII values indicate that no significant interactive effect was observed for *I. pseudacorus*, but that the presence of *I. pseudacorus* facilitated an increase in the total and above-ground biomass accumulation of *T. capensis*. While the RGR analysis suggests that *T. capensis* may be a superior competitor over *I. pseudacorus*, the lack of influence *T. capensis* holds over the growth of *I. pseudacorus* may suggest otherwise. As these results appear to be conflicting, longer-term competition experiments may provide greater insight into the interactions occurring between the species.

As the dense mats produced by *I. pseudacorus* rhizomes contribute to the invasiveness and negative environmental impacts posed by the species (Thomas, 1980; Lamote et al., 2002), it may be wise to conduct longer-term competition experiments when assessing the competitive ability of *I. pseudacorus*, allowing for the production of sufficient below-ground biomass, and the inclusion of multiple plant-life stages. The competitive abilities of plant species are known to change throughout the species' various life stages (Zhang and Lamb, 2012), and it is likely that the competitive interactions occurring between *I. pseudacorus* and *T. capensis* at the colonizing stage will differ to those occurring once the plants have established and matured. Furthermore, future studies aimed at assessing the competitive interactions between *I. pseudacorus* and co-occurring species should include an investigation into potential priority effects, as this would be more representative of field conditions (Alford and Wilbur, 1985; Geange and Stier, 2010; Fukami, 2015). While the experiment was intended to run for a period of one year, this was not possible due to the restrictions on movement posed by COVID-19, and so the experiment was concluded after six months. This likely affected the result obtained, as more mature populations of *I. pseudacorus* are displacing *T. capensis* populations at many invaded sites across South Africa (pers. obs). However, prolific growth of *T. capensis* has also been observed in the presence of *I. pseudacorus*, with certain conditions, life stages and/or priority effects likely influencing the competitive abilities of the different species in different ways.

A separate concurrent experiment was set up to assess the competitive interactions between *I. pseudacorus* and *Pontederia cordata*, but unfortunately the majority of *P. cordata* individuals did not survive the COVID-19 lockdown period as they were without water, and so insufficient plant material was available for inclusion in the experiment. However, the competitive interactions between *I. pseudacorus* and *P. cordata* were investigated in an honours research project in 2018, the results of which suggest *I. pseudacorus* was a superior competitor over *P. cordata*.

2.4.4 Conclusion

I hypothesised that *I. pseudacorus* would have a wider distribution with greater population densities in regions with warmer, wetter climates, as well as in major urban hubs. While the number of *I. pseudacorus* records was greatest in major urban hubs, the plant abundance in urban populations was not particularly high. Contrarily, the warmer, wetter regions of the country (Limpopo and Mpumalanga) contained populations of high abundance, but infestation records in these regions were much lower than that of the major cities, with temperature and precipitation influencing *I. pseudacorus* distributions. As expected, a high sexual reproductive output was observed in South African *I. pseudacorus* populations. Lastly, *I. pseudacorus* was expected to demonstrate superior competitive abilities over *T. capensis*, but this was not the case. However, longer-term competition experiments including multiple plant life stages may yield different results, as *I. pseudacorus* is not a particularly fast-growing weed. This chapter provided insight into the invasion autecology of *I. pseudacorus* in South Africa. Investigating the population genetics of South African *I. pseudacorus* infestations will further our understanding of the species invasion biology and ecology, enhancing the potential for effective management and control.

Chapter 3: Population genetics of *Iris pseudacorus* in South Africa

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3.1 Introduction

Population genetics is the study of the amount and distribution of genetic variation present between and within populations (Le Roux and Wieczorek, 2009). This variation may be due to changes or mutations at the molecular level, for example through the recombination of chromosomes or the insertion or deletion of DNA sequences (Le Roux and Wieczorek, 2009), and can provide a great deal of information regarding the autecology of the species in question.

A wide range of molecular techniques have been developed to address a multitude of questions posed by ecologists. The advancements in this technology and the increasing use of such techniques has enhanced many scientific fields, including invasion biology (Nei, 1975; Le Roux and Wieczorek, 2009; Gaskin et al., 2011). With an understanding of a population's biology at the molecular level, ecologists are able to investigate processes such as reproduction, dispersal, gene flow and hybridization in much greater detail, and gain a deeper understanding of population structure (Gaskin et al., 2011). Similarly, molecular ecology has enabled invasion biologists to better understand introduction events, match invasive populations to source populations, and investigate how invasive species may be spreading (Nei, 1975; Le Roux and Wieczorek, 2009; Gaskin et al., 2011).

3.1.1 Population genetics and invasion ecology

The increased application of molecular techniques in invasion biology and ecology has provided much insight into the mechanisms involved in species invasions (Lee, 2002). As the success of invasions depends largely on a species ability to adapt to a given environment, having the technology to investigate such adaptation on a genomic level has proven to be of great value. Through the use of molecular techniques, invasion biologists can disentangle complex taxonomical issues, investigate the geographical sources of invasive populations, and monitor their spread across a given landscape (Le Roux and Wieczorek, 2009).

Using molecular markers, it is possible to identify and track adaptations on a genomic level which may not be phenotypically observable. These adaptations can arise through novel mutations, or through the recombination of genes during meiosis in sexual reproduction, provided genetic variation was pre-existing between parent organisms (Bock et al., 2014). Genetic variation may occur through the insertion or deletion of DNA sequences, the inversion or rearrangement of DNA segments, and through the substitution of single nucleotide bases (Le Roux and Wieczorek, 2009). This variation can be tracked through DNA-based molecular markers, which can aid in elucidating the dispersal patterns of an invader, providing a better understanding of its reproductive ecology (Le Roux and Wieczorek, 2009).

As the genetic diversity of invasive (or alien) weed populations is representative of only introduced genotypes, these populations tend to exhibit lower genetic diversity than is the case in the native range (Paterson et al., 2009). This is especially true for asexually reproducing weeds, as genetic diversity can no longer arise through recombination. Sexually reproducing weeds, however, will demonstrate a substantially higher degree of genetic diversity (Paterson et al., 2009). Using molecular methods to analyse the levels of genetic diversity within and between populations of invasive weeds can provide useful insight into the reproductive ecology of the species in question. For example, one may be able to infer the species' relative employment of sexual vs asexual modes of reproduction, thus aiding control organisations in successfully managing invasive populations.

Genetic variation resulting from introgression and hybridization is another important factor to consider in invasion ecology, for which molecular-based techniques provide a much deeper understanding (Le Roux and Wieczorek, 2009). Without such techniques, ecologists would rely on phenotypic or morphological evidence, which may not be present or particularly evident. Both hybridization and genetic introgression can have major implications for species invasions (Ellstrand and Schierenbeck, 2000; Le Roux and Wieczorek, 2009). By masking deleterious alleles and both transferring and magnifying favourable alleles, the population may increase its ecological fitness, ultimately enhancing its invasive potential (Abbott, 1992; Le Roux and Wieczorek, 2009).

Hybridization of invasive species with native species poses the additional risk of displacing the native species from the invaded landscape, potentially leading to the extinction of the distinct native genotype (Huxel, 1999; Vilà et al., 2000; Muhlfeld et al. 2009). This displacement promotes biodiversity loss and can have knock-on effects on the ecological functioning of the invaded landscape (Vilà et al., 2000). For example, if the native genotype is more efficient at fulfilling certain ecological roles than the novel hybrid species which displaced it, the ecological structure of the system will be affected, and the functionality of the system may be impeded upon.

3.1.2 Population genetics and invasive species management

Invasion biologists and management organisations involved in the biological control of invasive species greatly benefit from the increased application of molecular techniques, which can reduce the risks associated with biological control and simultaneously increase its effectiveness (Gaskin et al., 2011). As taxonomic uncertainty can negatively affect the success of biological control programmes, using molecular-based approaches to resolve these uncertainties will allow for more effective management of invasive populations (Gaskin et al., 2011; Reid et al., 2021).

With a better understanding of the taxonomy of the target invader, it may be possible to match invasive populations to native source populations, facilitating the identification of appropriate potential biological control agents to be collected for further testing (Gaskin et al., 2011; Reid et al., 2021). Natural enemies often perform better on genotypes from their own local populations (Mopper and Strauss, 1998; Cory and Myers, 2004), and may demonstrate reduced fitness on genotypes with which they are not locally adapted (Goolsby et al., 2006; Gaskin et al., 2011; Paterson et al., 2012). This appears to be more common in weeds with high genetic diversity, but does not hold true for all host-enemy associations (Gaskin et al., 2011; Paterson et al., 2012). This has important implications for biological control, and the efficacy of biological control programmes can often be improved by collecting locally adapted natural enemies from the source of the invasion.

Molecular techniques have proven useful in elucidating aspects of the reproductive biology of invasive species. As the desired outcome of a biological control programme is to reduce the spread and density of an invasion, an understanding of how the species is reproducing and spreading is essential to effectively control invasive populations (Gaskin et al., 2011). Many invasive weeds reproduce both sexually (through the production of seed) and asexually (through vegetative fragmentation), and so identifying the primary mode of reproduction employed by the species would allow for the most appropriate guild of biological control agent to be selected (Gaskin et al., 2011). By gaining a better understanding of the species reproductive biology, control organisations can target the most relied upon reproductive structures of the plant, reducing their reproductive output more effectively, and controlling their invasions with greater success (Gaskin et al., 2011).

The hybridization of invasive species with native or invasive counterparts can have serious implications on their management and control (Zalucki et al., 2007; Reid et al., 2021). If hybridization increases the fitness of the species and its invasive potential is enhanced, management and control efforts may become more complicated and less successful. The effects of plant hybridization on insect herbivory vary, with some studies demonstrating decreased palatability to non-adapted herbivores, and other studies showing increased palatability of plant material due to a reduction in plant defences (Fritz et al., 1999; Zalucki et al., 2007; Reid et al., 2021).

3.1.3 Molecular techniques in population genetics

Many molecular techniques are available for use in population genetics studies and biological control programs, with the suitability of the technique dependent on the desired application and the availability of resources (Le Roux and Wieczorek, 2009; Gaskin et al., 2011). Molecular markers allow for the study of gene flow, and their development has advanced many fields with which population genetics is involved (Ouborg et al., 2008). The markers commonly utilised in population genetics studies include random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP) and simple sequence repeats (SSRs), also referred to as microsatellites (Ouborg et al., 2008).

Simple sequence repeats (SSRs) are short series of DNA nucleotide units, which are repeated in tandem throughout the genome of a species (Hamada et al., 1982; Powell et al., 1996). These markers demonstrate high levels of polymorphism, and are considered amongst the most informative molecular markers available for use in population-level genetics studies (Abbot, 2001; Le Roux and Wieczorek, 2009). Inter-simple sequence repeats (ISSRs) amplify the regions between neighbouring SSRs using a single anchored primer, and are advantageous in that they require no prior knowledge of the species' genome (Wolfe et al., 1998; Le Roux and Wieczorek, 2009). Furthermore, ISSRs exhibit high levels of polymorphism, are relatively quick and easy to apply, have high reproducibility, and can reveal small-scale variation between and within populations at a reasonable cost, making them particularly useful in intraspecific population genetics studies (Le Roux and Wieczorek, 2009; Paterson and Zachariades, 2013).

3.1.4 *Iris pseudacorus* population genetics

At present, the genetic and geographical origins of invasive *I. pseudacorus* populations is not known (Minuti et al., 2021), but a handful of studies regarding the species population genetics and degree of genetic variation are available (Lamote et al., 2002; Gaskin et al., 2016). As an invasive aquatic plant, *I. pseudacorus* has been expected to demonstrate low levels of genetic variation, as many aquatic angiosperms primarily reproduce asexually. However, both Lamote et al. (2002) and Gaskin et al. (2016) found populations of *I. pseudacorus* to be more genetically diverse than previously anticipated, suggesting the species may employ sexual modes of reproduction to a much higher degree than was thought to be the case. While Lamote et al. (2002) observed distinct grouping patterns in Bulgarian *I. pseudacorus* populations as a result of geographic barriers, their results suggest that both sexual and asexual reproduction is occurring in each of the separate populations. Gaskin et al. (2016) reports that *I. pseudacorus* populations in the northwest USA reproduce almost solely by seed, with 98 % unique genotypes observed throughout the invasion.

3.1.5 Aims and hypotheses

This chapter aimed to i) determine the genetic diversity present within and between South African *I. pseudacorus* populations; ii) investigate the primary mode of reproduction employed by South African *I. pseudacorus* individuals; and iii) investigate the potential effect of geographical distance on genetic diversity. This information will allow for the most appropriate control measures to be taken against *I. pseudacorus* invasions in South Africa, forming part of the global south initiative to control invasive *I. pseudacorus* populations. An understanding of the reproductive ecology and genetic diversity present in South African *I. pseudacorus* populations will aid control organisations by enabling appropriate biological control agents to be sourced, and for the appropriate reproductive structure of the plant to be targeted.

It is hypothesised that i) a high level of genetic diversity will be observed within and between South African *I. pseudacorus* populations; ii) sexual reproduction will be identified as the primary mode of reproduction employed by South African *I. pseudacorus* populations; and iii) genetic diversity will not be affected by geographical distance.

3.2 Materials and Methods

3.2.1 Fieldwork

Iris pseudacorus samples were collected from 21 sites in 7 of South Africa's 9 provinces (Table 2.1). At each site, leaf tips were collected from individuals at least 5 m apart so as to avoid sampling clonal plants. Where possible, 10 leaf tips were collected per site. However, as some infestations were relatively small, this was not always possible. At these sites, as many leaf tips as possible were collected abiding to the sampling distance of 5 m. Where infestations were particularly widespread, the sampling distance was increased to 10 m to ensure the entire range of genetic variation was included in the study. Each leaf sample was dried with tissue paper and placed into individual paper envelopes. The paper envelopes from each of the sites sampled were placed into Ziploc bags containing silica gel, and were stored in cool, dry conditions in the laboratory.

3.2.2 DNA extractions

Each of the 145 leaf samples collected from the 21 sites were ground into a fine powder (approx. 30-40 mg) using liquid nitrogen in a mortar and pestle. The DNA was then extracted using QIAGEN's DNeasy Plant Mini Kit (QIAGEN Inc.). The extracted DNA was stored in Eppendorf tubes at -20 °C until required for further use.

3.2.3 ISSR PCR protocol

Preliminary tests to identify suitable primers were conducted, and the universal primer HB15 was selected based on the number of peaks produced. The primer was labelled with 6-FAM fluorescent dye at the 5' end by manufacturers at Applied Biosystems Inc., U.K. (Barker et al., 2015).

Each PCR reaction comprised the following volumes and concentrations of reagents: 0.8 µM of HB15 primer, 10 µL of iTaq™ Universal SYBR® Green Supermix (Bio-Rad), 3 µL of DNA, and 6.2 µL denucleated water. Following the methodology described in Paterson et al. (2009) and Barker et al. (2015), the PCR protocol began with a denaturing step of 2 min at 94 °C, followed by 35 cycles of 94 °C for 30 s, 44 °C for 45 s, and 72 °C for 90 s and a final extension of 20 min at 72 °C. Each reaction was replicated to ensure reproducibility.

3.2.3 Electropherograms

The PCR products were sent to the Central Analytical Facility (CAF) at Stellenbosch University, where capillary electrophoresis fragment analyses were conducted using a LIZ1200 genetic analyser to produce a binary matrix based on the presence or absence of bands. The electropherograms were analysed using GeneMarker ver. 2.7.4 (SoftGenetics, LLC), and RawGeno ver. 2.0 (Arrigo et al., 2009), which was run through R ver. 4.0.3 (R Core Team, 2020), was used to score the datasets. Only the electropherogram peaks present in both duplicates were included and scored.

3.2.4 Scoring parameters

Scoring parameters were selected based on recommendations by Arrigo et al. (2012), as well as their performance in preliminary tests, with those producing the lowest error rates being chosen. A minimum peak height of 50 RFU was selected in GeneMarker, with both the AFLP normalization and stutter peak filter unchecked, and 'smoothing' selected. The data were imported into RawGeno, in which the minimum and maximum bin widths were set at 1 and 1.5 respectively. The relatively small bin width of 0.5 has been shown to produce decent resolutions with minimised errors and is deemed reliable by Holland et al. (2008) and Arrigo et al. (2012). Peaks with sizes smaller than 100 bp and larger than 500 bp were discarded, as small peak sizes are considered more likely to be homoplasic, while relatively large peak sizes may not be detected consistently amongst individuals (Arrigo et al., 2012). The data were filtered further according to the number of peaks detected, with the upper and lower 5 % of data being removed, and individuals with fewer than 2 peaks discarded from the dataset).

The binary matrix generated by RawGeno was edited using Microsoft Excel and converted into a consolidated binary matrix using BINMAT: For Fragment Analysis Data (Clarke van Steenderen, https://clarkevansteenderen.shinyapps.io/BINMAT/). BINMAT further generated data summaries and average Euclidean and Jaccard's error rates, which were utilised alongside non-metric Multidimensional Scaling (nMDS) plots to determine optimal settings.

3.2.5 Data analysis

Once the optimal settings and scoring parameters were identified and the relevant filtering of data had occurred, 132 of the original 145 samples were included for further analysis. Using these data, non-metric Multidimensional Scaling (nMDS) plots were created using the consolidated binary matrix generated in BINMAT, allowing for data visualisation and identification of potential groupings and/or genetic diversity present. This was done using the programme PAST: Paleontological Statistics package ver. 4 (Hammer et al., 2001), which was also used to produce a pairwise similarity matrix from the binary matrix using Jaccard's similarity index, quantifying the genetic similarity between all individuals included in the matrix.

The pairwise similarity matrix produced was used to identify the number of unique genotypes present throughout the invasion. For each plant, 85 loci were scored, and identical individuals would have a Jaccard's similarity value of 1 (85/85). Plants which varied at one locus would have a similarity value of 0.988 (84/85), those which varied at two loci would have a similarity value of 0.976 (83/85), and so forth. The number of identical plant pairs (i.e. those with a similarity value of 1) were counted in Microsoft Excel, and the proportion of unique genotypes was determined (Gaskin et al., 2016).

SplitsTree ver.4.17.1 (Huson and Bryant, 2006) was used to construct an unrooted phylogenetic network using NeighbourNet construction and Jaccard's distances with 1000 bootstrap replication for node support (Reid et al., 2021). These analyses are more appropriate for intraspecific and population level phylogenetic studies than more traditional approaches (e.g., Bayesian analyses), as they consider and account for population-level phenomena to a greater degree (Posada and Crandall, 2001; Reid et al., 2021).

Using the Jaccard's similarity index produced in PAST, mean pairwise genetic distances were calculated for each site, and the mean within- and between-population genetic distances were compared using a Wilcoxon rank-sum test as the data did not follow a normal distribution. The effects of site on genetic similarity for both within- and between-population means were investigated using a Kruskal-Wallis ANOVA as the data did not follow a normal distribution. Following this, Dunn's Test was used to examine significant genetic differences. All statistical analyses were conducted using R ver. 4.0.3 (R Core Team, 2020).

The mean genetic and geographical distance between each population was calculated to determine whether genetic distance increases with geographical distance, providing information on the dispersal of genes between populations of *I. pseudacorus*. Genetic distance was calculated using the Jaccard's similarity matrix produced using PAST, while geographical distance was calculated using the ruler function in Google Earth Pro (v7.3). To determine whether any significant correlation exists between genetic and geographical distance in South African *I. pseudacorus* populations, Spearman's rank correlation coefficient test was conducted using R ver. 4.0.3 (R Core Team, 2020). Spearman's test was used as the data did not conform to a normal distribution.

Using a Bayesian clustering algorithm provided in STRUCTURE ver. 2.3.4 (Pritchard et al., 2000; Falush et al., 2003; Falush et al., 2007; Hubisz et al., 2009), individual samples were assigned to genetic clusters (K) based on the similarities between their genotypes. Following a slight adaptation of the methodology described in Gaskin et al. (2016) and Reid et al. (2021), the probability of an individual being assigned to a specific genetic cluster was determined using a 50,000 run burn-in with a Monte Carlo Markov Chain (MCMC) length of 100,000. Using StructureSelector (Li and Liu, 2018), the ΔK statistic (Evanno et al., 2005) and the

Peuchmaille Method for K selection (Puechmaille, 2016) were utilised to determine the optimal number of genetic clusters (K). CLUMPAK software (Kopelman et al., 2015) was used to produce graphical representations of the results obtained through STRUCTURE for the selected K values. A threshold of 0.8 was used, as larger thresholds utilise more stringent algorithms to assign individuals to specific cluster groups, and so the likelihood of assignment to an inappropriate cluster is reduced (Puechmaille, 2016).

3.3 Results

The final dataset, i.e. that in which low-quality samples were removed, produced an average of 10.08 replicable peaks (range = 2 to 23 peaks), with 85 loci scored. The final dataset yielded a mean Euclidean error rate of 7.76 % and a mean Jaccard error rate of 39.51 % based on the similarities and differences in peak presence or absence between the two replicates of each sample (Holland et al., 2008).

3.3.1 Unique genotypes

In the data matrix, which consisted of 8385 possible pairings, 165 identical plant pairs were found, and the proportion of identical genotypes was calculated as 165/8385 = 0.0197. Thus, the proportion of unique genotypes was calculated as 1-0.0197 = 0.980, or ~ 98 %. With a more conservative approach considered, if pairs which varied in up to 5 loci were considered 'identical' (i.e. those with similarity values < 0.941), only 5 additional plant pairs would be included, and ~ 97.8 % of genotypes would be considered unique.

3.3.2 Non-metric MDS

No clear clustering of samples was observed in the nMDS plot (Figure 3.1). As a stress value greater than 0.2 is considered close to random, it is likely that the pattern displayed by these data is also close to random. While no clear clustering exists, samples from the same sites are often relatively close together, indicating more genetic similarity within than between invaded sites (Figure 3.1). Overall, this suggests high genetic diversity between sites.

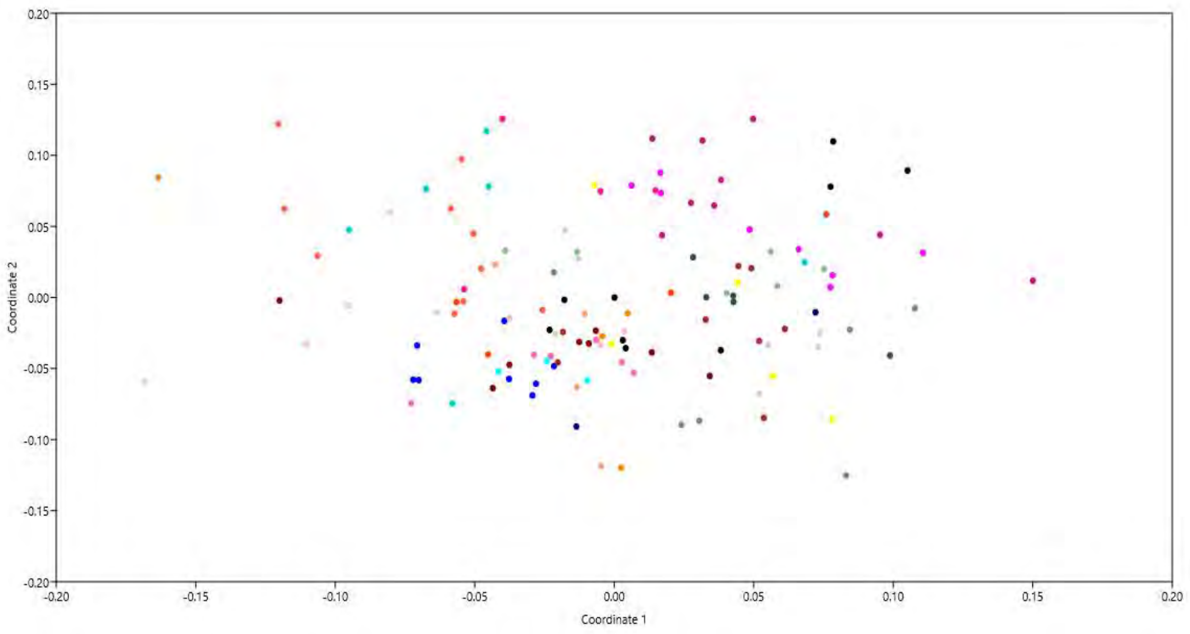


Figure 3.1: Non-metric MDS for 132 *Iris pseudacorus* individuals from 21 populations in South Africa, based on the Jaccard's similarity index. Different colours represent different sites. Stress = 0.296, R^2 axis 1 = 0.324, axis 2 = 0.2903.

3.3.3 NeighbourNet Tree

The NeighbourNet tree produced in SplitsTree suggested the presence of four potential genetic clusters (Figure 3.2). The *I. pseudacorus* populations in northern Limpopo (LKW and MGK) form an almost-exclusive cluster (blue), while the remaining three clusters are more variable (Figure 3.2). Most samples collected from Kwa-Zulu Natal group together (orange), but this cluster is not exclusive to Kwa-Zulu Natal, and includes samples from Mpumalanga, Gauteng, Limpopo, and the Western Cape (Figure 3.2). The majority of samples in the green cluster are from Johannesburg (WH, EG, ZL, GH), but samples from the North-West (GM), Mpumalanga (DK) and Kwa-Zulu Natal (LR) also form part of this cluster (Figure 3.2). The remaining (maroon) group is quite variable, with samples from Limpopo, Gauteng, the North-West, Kwa-Zulu Natal and the Western Cape included in this cluster (Figure 3.2) While these clusters can be observed based on their separation from other clusters in the NeighbourNet tree, clustering is not necessarily distinct, and inferences made from the NeighbourNet tree alone are not sufficient to suggest separate founding events or genetic drift (Figure 3.2).

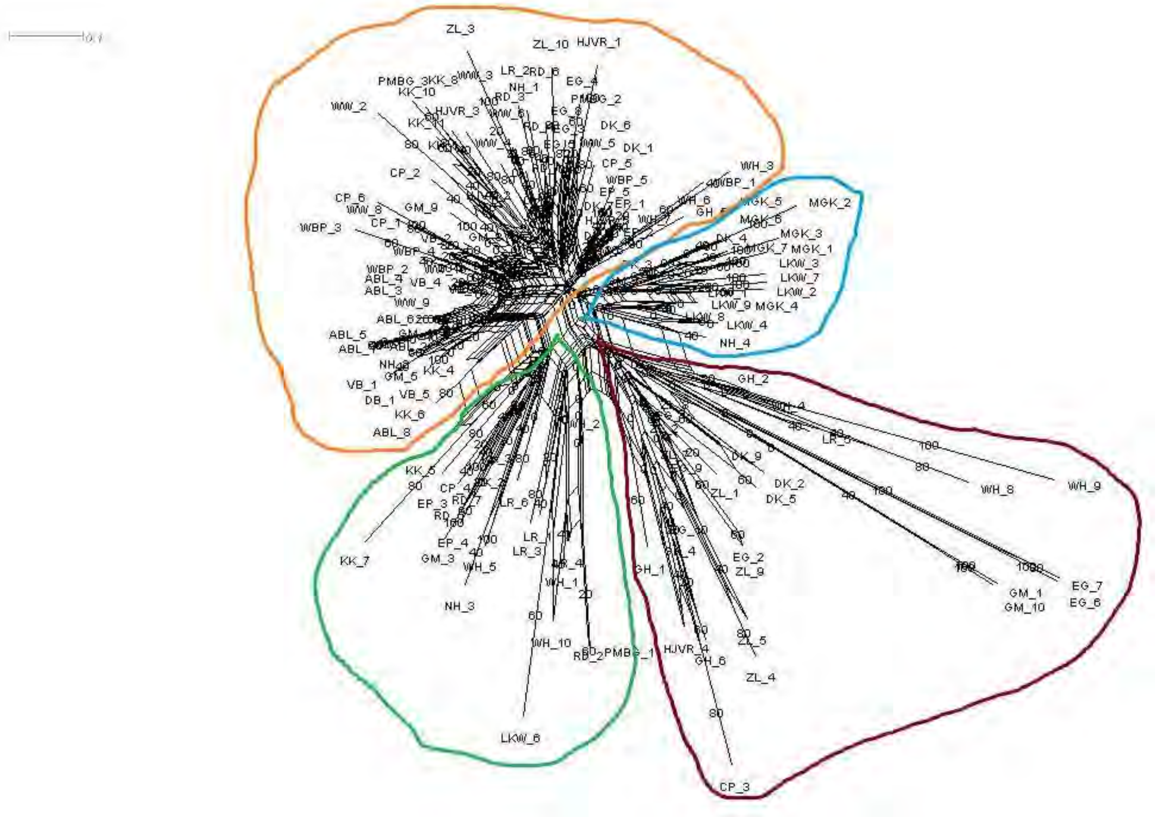


Figure 3.2: NeighbourNet tree produced in SplitsTree using Jaccard's distance. Different coloured outlines represent potential clusters according to genetic relatedness of individuals.

3.3.4 STRUCTURE analysis

Using StructureSelector, support was provided for K values of 4, 5 and 6 using the Puechmaille Method of K selection (Puechmaille, 2016), and a K value of 2 using the Evanno Method (Evanno et al., 2005). It is important to consider the biology of the organism being studied when selecting an optimal K value, and to be cautious when interpreting outputs provided by STRUCTURE (Pritchard, 2007; Gilbert et al., 2012). As such, considering the StructureSelector output and the grouping pattern shown in the NeighbourNet tree (Figure 3.2), K = 2, K = 3, and K = 4 are presented (Figure 3.3). While the plots produced with K values of 5 and 6 may provide further insight into the genotypic similarities between populations, interpreting plots becomes increasingly complicated with increasing values of K.

The Ascott Bush Lodge (ABL), Constantia Park (CP), Durban Botanical Gardens (DB), Groot Marico (GM), Wyneberg Park (WBP) and Vanderbijl Park (VB) populations grouped together, forming the 'orange cluster' (Figure 3.3), similar to the NeighbourNet tree cluster (Figure 3.2). While most GM samples formed part of the 'orange cluster', one sample fitted almost exclusively into the ‘purple cluster’, while another fitted almost exclusively into the ‘green cluster’ (Figure 3.3). While the VB population came close, no population fitted exclusively into any of the four clusters (Figure 3.3). In agreement with the NeighbourNet tree, the Emma Park (EP), Glenhazel Park (GH), Klein Kariba (KK), Lions River (LR) and Walkhaven Dog Park (WH) populations were largely assigned to the ‘green cluster’ (Figure 3.3). The ‘blue cluster’ comprised samples from many populations, most notably samples from the Dunkeld Country Estate (DK), Emmarentia Botanical Gardens (EG), Hoerskool Jan van Riebeck (HJVR), Haenertsburg (LKW), Rosette Village Dam (RD) and Walkerson’s Estate (WW) (Figure 3.3). Only one sample from the GM population fitted almost exclusively into the ‘purple cluster’ with both K = 3 and K = 4, followed by the Magoebaskloof (MGK), Haenertsburg (LKW) and KK populations (Figure 3.3). Overall, while some genetic clustering is evident, South African *I. pseudacorus* populations are quite genetically diverse, and it is likely that gene-flow is occurring both between and within populations (Figure 3.3).

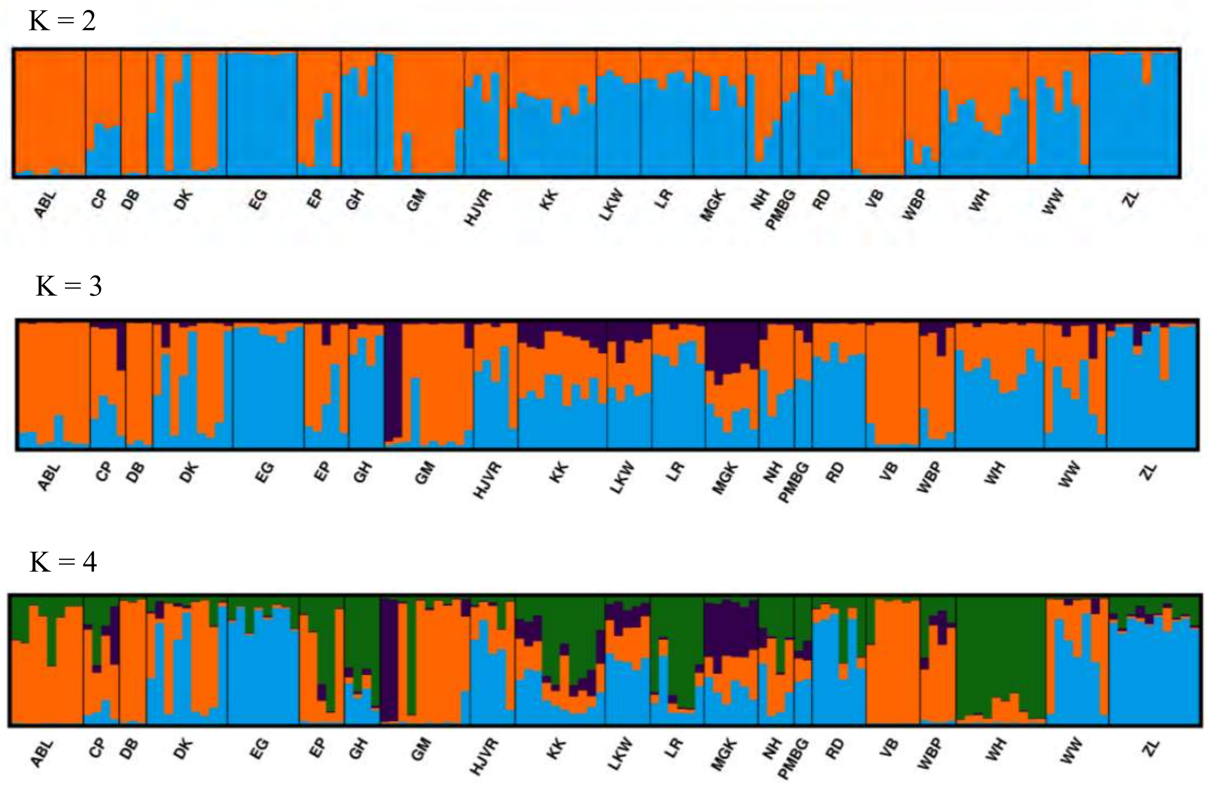


Figure 3.3: Results from STRUCTURE analysis of South African *Iris pseudacorus* populations. Site abbreviations defined in Table 2.1. Coloured bars represent genetic similarity of samples, and bar width represents sample size.

3.3.5 Genetic distance

A significantly greater degree of genetic similarity was observed within populations than between populations of *I. pseudacorus* (W = 9471354, $P < 0.0001$) (Figure 3.4). Sites with similarity indices close to 1 are genetically similar, while those with values closer to 0 are more genetically distinct.

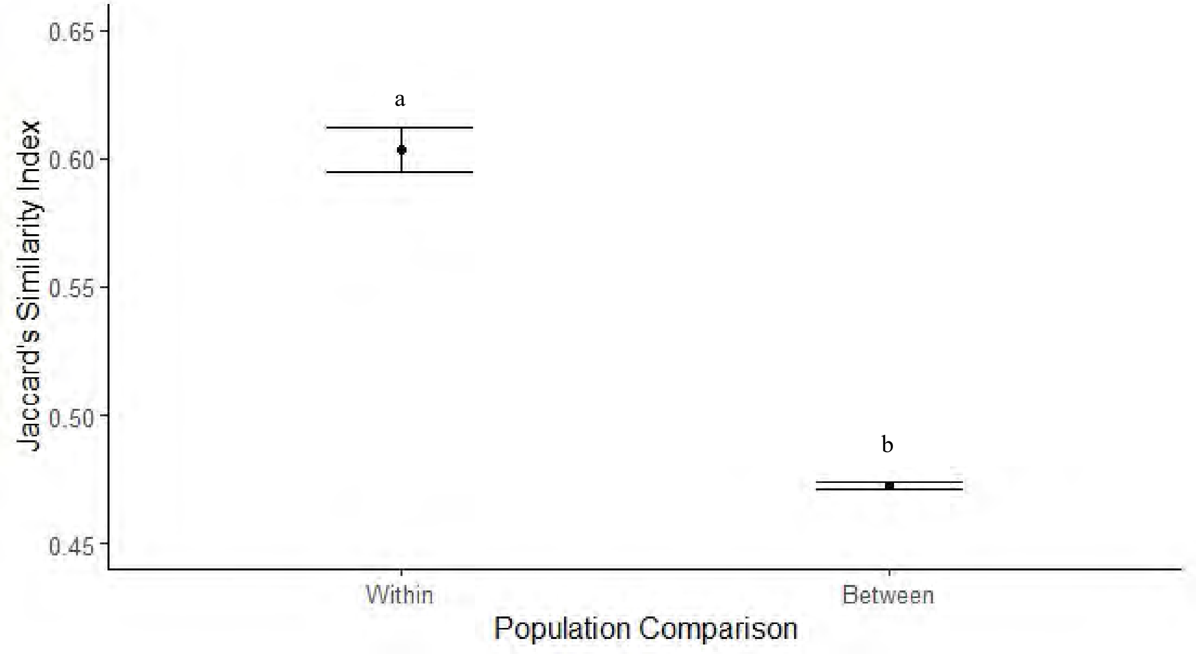
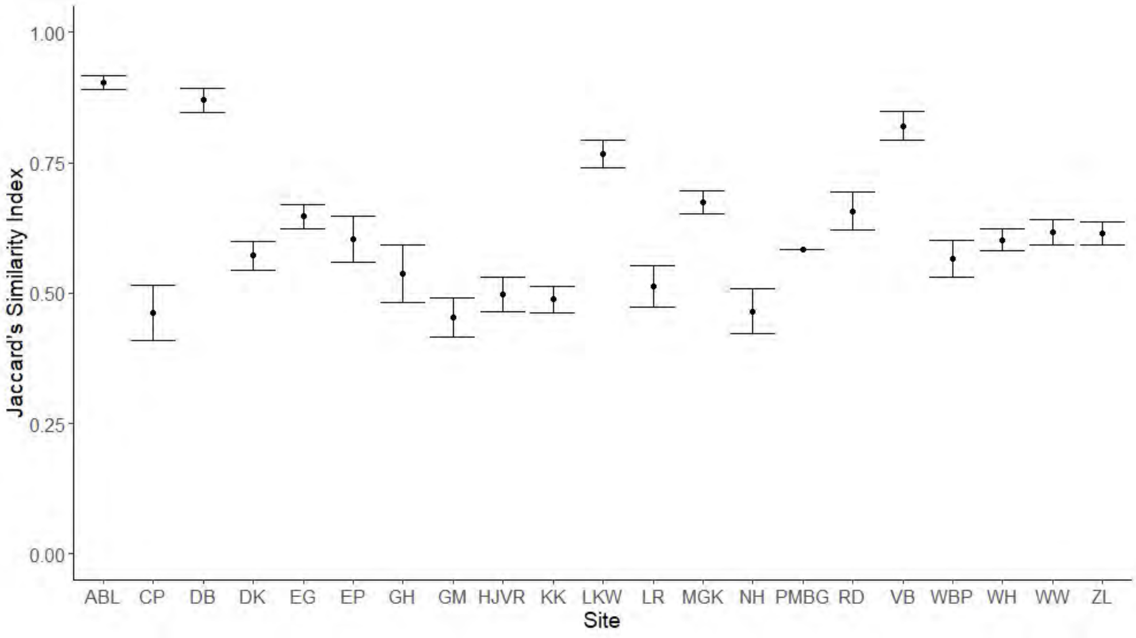
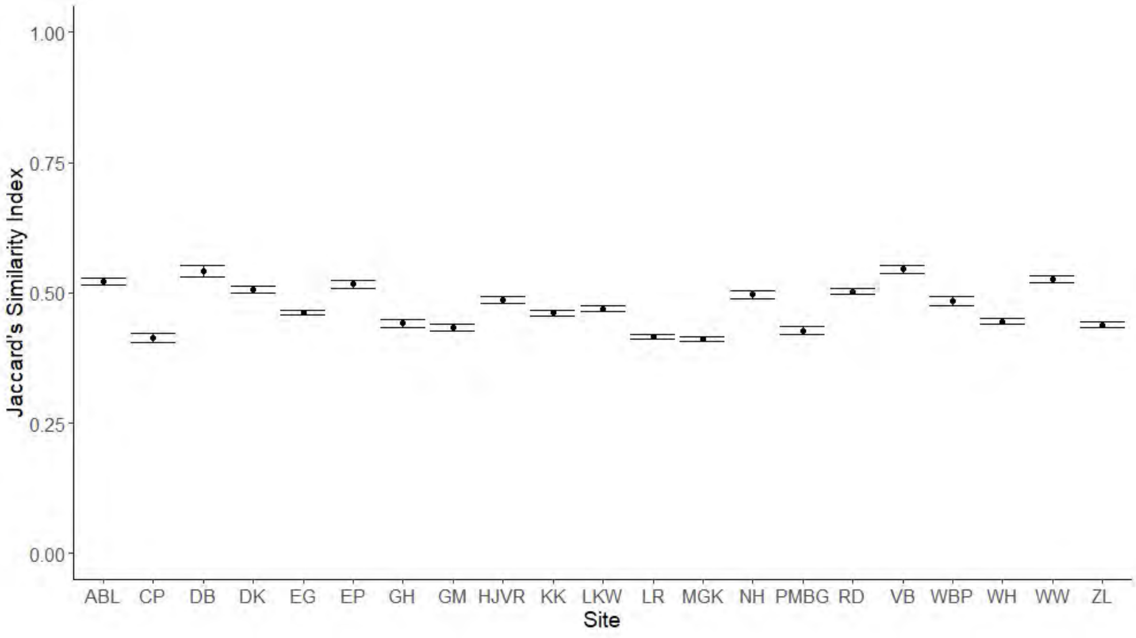


Figure 3.4: Comparison of mean (± S.E.) degree of genetic similarity within and between 21 South African *Iris pseudacorus* populations. Different letters denote significant differences between groups.

Site had a significant effect on genetic similarity in both the within- ($\chi^2 = 199.89$, df = 20, $P < 0.0001$) and between-population ($\chi^2 = 659.04$, df = 20, $P < 0.0001$) comparisons (Figure 3.5a, Figure 3.5b). More varied results were observed for the within-population comparison (Figure 3.5b) than was the case for the between-population comparison (Figure 3.5c), likely due to the exponentially larger sample size utilised in the between-population comparison.



a)



b)

Figure 3.5: Mean (± S.E.) a) within-, and b) between-population genetic similarity of 21 South African *Iris pseudacorus* populations

Despite the genetic variability between populations, a significant, albeit weak, negative correlation was observed between the mean geographical distance and the mean genetic similarity of *I. pseudacorus* populations in South Africa ($R = -0.21, P < 0.01$) (Figure 3.6). In other words, sites which were more geographically separate are less genetically similar (Figure 3.6).

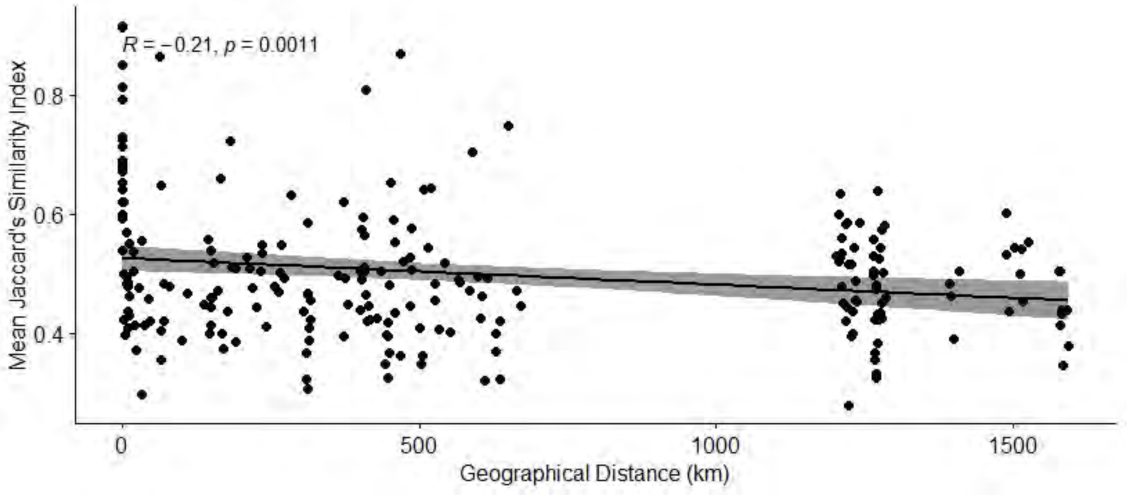


Figure 3.6: Correlation between genetic and geographical distance of 21 South African *Iris pseudacorus* populations using Spearman's rank correlation test

3.4 Discussion

A large amount of genetic diversity was observed both between and within populations of *I. pseudacorus* in South Africa. With approximately 97.8 – 98 % unique genotypes observed throughout the sampled populations, South African *I. pseudacorus* populations appear to reproduce largely through sexual means. This result is unusual for an invasive aquatic plant species, as the majority reproduce primarily through means of vegetative propagation (Barrett, 1980; Johansson and Nilsson, 1993; Gaskin et al., 2016). However, similar results were obtained for *I. pseudacorus* populations in the northern hemisphere (Lamote et al., 2002; Gaskin et al., 2016). While *I. pseudacorus* populations certainly do reproduce asexually, through the production and fragmentation of rhizomes, the genetic diversity and proportion of unique genotypes observed throughout the invaded range indicates that a large amount of geneflow is occurring between and within populations.

No clear clustering of samples was observed in the nMDS plot produced, suggesting high genetic diversity between and within populations, with geneflow likely occurring between invaded sites. While there were no clear clusters in the nMDS plot, identical colours are often relatively close together, suggesting more genetic diversity exists between than within populations. The NeighbourNet tree provides a clearer clustering pattern of four potential genetic clusters. However, these clusters contain individuals from various populations, and individuals from certain populations appear in different clusters. This supports the idea that gene flow has occurred between populations, contributing to the high genetic diversity. The STRUCTURE analysis supported the clustering pattern of the NeighbourNet tree, with support for a K value of 4 using the Puechmaille Method of K selection (Puechmaille, 2016). Furthermore, similar groupings of samples were observed between the NeighbourNet tree and the STRUCTURE analysis, each of which supporting the other.

While grouping of samples is evident in both the NeighbourNet tree and the STRUCTURE analysis, the absence of clear clustering in the nMDS plot suggests the data are insufficient to infer separate founding events and/or genetic drift thereafter, unlike the results obtained by Gaskin et al. (2016), which found support for separate founding events. The inclusion of native-range genotypes in future studies could identify source populations from which biological control agents could be sourced. This could enhance the efficacy of biological control programmes by reducing the potential for host plant incompatibilities, and by ensuring the candidate biological control agent is well adapted to the invasive genotype (Paterson et al., 2009; Gaskin et al., 2011; Barker et al., 2015; Sutton et al., 2017). As these results do not suggest multiple founding events, this may improve the prospects for biological control of *I. pseudacorus* invasions in South Africa.

A significantly greater amount of genetic diversity was observed between populations than within populations of *I. pseudacorus* in South Africa. Samples from the same populations of *I. pseudacorus* were more genetically similar, indicating that both sexual and asexual reproductive strategies are employed within populations. Furthermore, as site affected the genetic similarity of samples both within- and between-populations, different populations of *I. pseudacorus* appear to employ sexual and asexual reproductive strategies to varying degrees, with sites such as Ascott Bush Lodge (ABL), the Durban Botanical Gardens (DB), Haenertsburg (LKW), Magoebaskloof (MGK) and Vanderbijl Park (VB) reproducing largely through vegetative means.

The negative correlation between geographic distance and genetic similarity indicates that samples which have a greater geographical distance between them are less genetically similar. While this correlation was statistically significant, the correlation was weak, suggesting that geneflow is still occurring between geographically distant populations, but to a lesser degree than populations which are closer together. This supports the artificial establishment of populations, with horticulturalists and gardening enthusiasts sharing seed or vegetative fragments. The large gap in geographic distance is due to the proximity between samples collected from Cape Town in the Western Cape and samples collected further east. The presence of a correlation, albeit a weak one, may suggest fewer founding events in South Africa than is the case in the United States, where the lack of correlation between geographic and genetic distance amongst *I. pseudacorus* populations is the result of multiple randomized founding events across the sampled landscape (Gaskin et al. 2016).

When assessed together, these results indicate that *I. pseudacorus* reproduces sexually and asexually, with different proportions of each reproductive strategy between populations. Interestingly, while significantly fewer flowers were produced amongst populations in the Western Cape Province (see Chapter 2), these populations did not display particularly high levels of genetic similarity. As no environmental data were collected in this study, it is not possible to draw any firm conclusions as to what may lead to the prevalence of either reproductive strategy.

The high level of genetic diversity coupled with the high germination rate reported in Chapter 2 of this thesis indicate that invasive populations of *I. pseudacorus* in South Africa behave similarly to the invasive populations studied by Gaskin et al. (2016) in the United States. It would be interesting to compare these results with those of other invaded ranges, such as Argentina and New Zealand, as well as with those of native *I. pseudacorus* populations. Through collaborative efforts, these data should be available in the near future, after which further investigation into the autecology of *I. pseudacorus* may occur.

These results may aid in the monitoring, management, and control of *I. pseudacorus* populations in South Africa. As South African *I. pseudacorus* populations reproduce both sexually and asexually, both clonal and sexual reproductive structures must be targeted when attempting to control or eradicate populations. As such, the timing of both manual and chemical control should be such that seed development is prevented (Gaskin et al., 2016). Furthermore, should biological control be applied to *I. pseudacorus* infestations, more than one biological control agent should be selected, such that the production of both sexual and asexual propagules is reduced.

Chapter 4: General discussion

4.1 Introduction

This thesis aimed to understand the invasion biology and autecology of *I. pseudacorus* populations in South Africa. By investigating the species' distribution, abundance, sexual reproductive output, competitive ability, and population genetics, this study contributes to the global south initiative aimed at controlling *I. pseudacorus* invasions in the southern hemisphere.

Through field surveys, the distribution, abundance, and sexual reproductive output of *I. pseudacorus* populations were determined, and the potential effects of climate investigated. The majority of infestation records came from the country's major cities, while the most severe infestations were recorded in Limpopo and Mpumalanga. The severity of invasion appears to be linked to temperature and precipitation, but as *I. pseudacorus* appears to be in the "lag" phase of its invasion in South Africa (Blackburn et al., 2011), the data were insufficient to draw firm conclusions. The sexual reproductive output of South African *I. pseudacorus* populations has been enhanced relative to native range populations, with a germinability of ~ 83 %, and a substantially greater number of seeds produced than that reported in the Netherlands (Coops and Van der Velde, 1995). Unexpectedly, *T. capensis* outperformed *I. pseudacorus* in the competition experiment, with the growth of *T. capensis* increasing in the presence of *I. pseudacorus*, indicating a facultative interaction (Balestri et al., 2021). The short duration of the experiment may have affected these results, as *I. pseudacorus* demonstrated a high RSR and its growth appeared unaffected by the presence of *T. capensis*.

DNA samples collected during field surveys were used to determine the amount of genetic diversity present between and within South African *I. pseudacorus* populations, to investigate the relative employment of sexual vs. asexual reproductive strategies, and to assess the effects of geographic distance on genetic similarity. There was a high level of genetic diversity between and within populations, with ~ 98 % unique genotypes observed. The results show that gene-flow has occurred, and so it is likely that South African *I. pseudacorus* populations reproduce largely through sexual means. While this result is unusual for an invasive aquatic species, similar results were found in Belgium (Lamote et al., 2002) and in the United States (Gaskin et al., 2016). Unlike the findings of Gaskin et al. (2016), a negative correlation was observed between geographic distance and genetic similarity, suggesting the occurrence of fewer founding events in South Africa than was the case in the United States. However, this correlation was weak, indicating a largely artificial (or anthropogenic) spread of *I. pseudacorus* populations in South Africa.

These results should be included in the development of monitoring, and management plans for *I. pseudacorus* infestations in South Africa, and contribute to the global south initiative to mitigate and control *I. pseudacorus* invasions throughout the southern hemisphere. As such, the implications of these findings are discussed in a South African and southern hemisphere context.

4.2 Implications for South Africa

4.2.1 Invasion of *I. pseudacorus* in South Africa

South Africa's freshwater systems are amongst the most eutrophic in the world, supporting invasion by a variety of aquatic macrophytes (Coetzee and Hill, 2012). As these waters are nutrient-rich, aquatic invasions are no longer limited by resource availability. Coupled with their 'escape' from suppression by natural enemies, this increase in resource availability has allowed for the rapid proliferation of invasive populations in South Africa's freshwater environments.

Many of South Africa's aquatic invaders were introduced through horticultural activities and the aquarium trade, including water hyacinth (*Eichhornia crassipes* (Mart.) Solms-Laubach (Pontederiaceae)) for the former and parrot's feather (*Myriophyllum aquaticum* (Vell.) Verdc. (Haloragaceae)) for the latter (Martin and Coetzee, 2011). While a vast number of species are introduced outside of their native ranges each year, only a small portion of these species become problematic, and those which do often experience substantial time-lags between their introductions and their subsequent invasions (Blackburn et al., 2011; Rouget et al., 2015; Hinz et al., 2019). Controlling invasions during or prior to these time-lags will greatly increase the effectiveness of the control programme, while significantly reducing the environmental and economic costs associated with the invasion (Reaser et al., 2020). While it may be difficult to predict which introduced species will become problematic, investigating the species' invasion dynamics elsewhere often provides useful insight into its invasive potential in the landscape of interest. For example, the severity of invasion by *Hydrilla verticillata* (L.f.) Royle (Hydrocharitaceae) in the United States prompted a rapid response from South Africa to contain and control the weed when it was observed in 2004 (Coetzee et al., 2011b).

Iris pseudacorus infestations in South Africa appear to be in the “lag” phase of their invasion (Blackburn et al., 2011), with the country’s infestation records comprising many ‘artificial’ or cultivated populations. Nonetheless, *I. pseudacorus* has a broad distribution in South Africa, with confirmed infestations in all provinces except the arid Northern Cape. This is concerning, as *I. pseudacorus* infestations elsewhere, such as in Argentina (Figure 4.1), suggest the species is capable of outcompeting indigenous vegetation in favour of dense *I. pseudacorus* monocultures. As was the case for *H. verticillata*, a rapid response to contain *I. pseudacorus* invasions should be implemented before they reach the severity of infestation observed elsewhere.

While many reported infestations have yet to ‘escape’ cultivation, the relatively high abundance of *I. pseudacorus* individuals in South Africa’s more severe infestations (Figure 4.2), coupled with the findings of Minuti et al. (2022), caution that South Africa is certainly not immune to further invasion by *I. pseudacorus*. As new populations continue to be established by those unaware of the negative ecological impacts of invasive *I. pseudacorus* populations, preventing ‘artificial’ establishment of potential sources of further invasion is an important step in mitigating the environmental impacts of *I. pseudacorus* in South Africa. Anthropogenic factors appear to influence the current distribution of *I. pseudacorus* in South Africa to a greater degree than climatic factors, as is often the case in the early stages of invasion by exotic species (Barney, 2006). However, the absence of infestations in the country’s more arid regions and the establishment of dense populations in the warmer, more humid regions of the country indicate that climate certainly will influence the invasion dynamics of South Africa’s *I. pseudacorus* infestations.

Figure 4.1: *Iris pseudacorus* invasion in Buenos Aires Province, Argentina (photo by A. Faltlhauser, 2021)

Figure 4.2: *Iris pseudacorus* invasion in Haenertsburg, Limpopo, South Africa (photo by J. Coetzee, 2020)

Invasive *I. pseudacorus* populations in South Africa have high sexual reproductive capacity, producing vast numbers of highly germinable seed towards the end of summer. This finding is common amongst invasive species (Scott, 1996; Buckley et al., 2003; Correia et al., 2016;

Daws et al., 2007), and is likely a result of the ERH, or 'escaping' the regulation provided by a suite of co-evolved natural enemies in the species native range (Keane and Crawley, 2002). This 'escape' may have allowed for a shift in defence strategy, increasing the availability of resources to be allocated to growth, competition, and reproduction, as postulated in the EICA hypothesis (Joshi and Vrieling, 2005). Evidence to support the ERH has been found for *Sagittaria platyphylla* (Engelm.) J.G.Sm. (Alismataceae), an incipient invader in South Africa (Kwong et al., 2019), for which early detection and rapid response programmes were initiated (Ndlovu et al., 2020).

The potential reallocation of resources in the absence of natural enemies has enhanced the invasive potential of South African *I. pseudacorus* populations. As invasive potential depends largely on effective reproduction, propagation, and dispersal (Barrett et al., 2008; Gaskin et al., 2016), the enhanced production of highly germinable seed will likely promote the invasion of *I. pseudacorus* in South Africa, while increasing the complexity associated with its control. Increased propagule production and release (i.e. 'propagule pressure') will provide more opportunities for the spread and establishment of novel *I. pseudacorus* populations, and the potential seed bank produced will further complicate control efforts. Furthermore, the increase in genetic diversity associated with an increased sexual reproductive output may complicate control efforts, owing to the potential for herbivore-host maladaptation (Paterson et al., 2014), as well as the potential for hybridization (Reid et al., 2021).

Following the dispersal of viable propagules, an invasive species must compete with co-occurring biota in the invaded range in order to establish and maintain a viable population. With interspecific competition being amongst the most important factors in determining the likelihood of a successful invasion (Crawley, 1990), the competitive edge *T. capensis* exerted over *I. pseudacorus* may prove beneficial in controlling *I. pseudacorus* populations at sites in which *T. capensis* is present. However, field observations do not necessarily support this result, as a gradual replacement of *T. capensis* by *I. pseudacorus* was observed at some invaded sites (e.g. Glenhazel Park, Johannesburg). As plant life stage and priority effects play large roles in determining the competitiveness of a species, the inclusion of multiple life stages and a range of priority effects would provide more insight into the competitive abilities of *I. pseudacorus* in South Africa.

While it was not possible to explore the competitive interactions occurring between *I. pseudacorus* and another wetland invader, *P. cordata* in this study, the results obtained from an honours research project in 2018 suggest *I. pseudacorus* is a superior competitor over *P. cordata*. This result is both supported and contested by field observation, with some sites indicating the suppression of *I. pseudacorus* populations by co-occurring *P. cordata* populations, and other sites the opposite effect. Investigating the factors that influence the competitive ability of *I. pseudacorus* may aid control by identifying high-risk systems, and by using appropriate native competitors to suppress *I. pseudacorus* infestations. Considering *I. pseudacorus* has only recently become invasive in South Africa, investigating potential priority effects may provide great insight into the invasive potential of *I. pseudacorus* populations.

4.2.2 The control of *I. pseudacorus* invasions in South Africa

Controlling invasive species in the early stages of their invasions increases control success and mitigates the environmental and economic costs associated with the invasion (Reaser et al., 2020). Early response programmes are most successful when target populations are contained, and become increasingly challenging and costly when infestations become more widespread (Ndlovu et al., 2020). When invasions become extensive, resources become diluted, and eradication becomes less likely, as experienced with the attempted management of South Africa's *S. platyphylla* invasions (Ndlovu et al., 2020).

To avoid further environmental and economic losses associated with *I. pseudacorus* invasions in South Africa, it is important that existing invasions be controlled, and further invasions be prevented. As many of South Africa's *I. pseudacorus* infestations are 'artificial' and not particularly severe, controlling these populations mechanically may prove effective, provided it is done before the species 'escapes' cultivation or before the infestations form dense stands and produce a prolific seed bank. However, in the country's more severe invasions, such as that in Haenertsburg, Limpopo (Figure 4.2), mechanical removal of *I. pseudacorus* will likely prove less effective, as the species produces dense stands and a vast number of germinable seed.

While large-scale invasions may be manageable through chemical control, no herbicide has been registered against *I. pseudacorus.* Biological control may prove effective in controlling the country's more severe invasions, and efforts are underway to develop a biological control programme for *I. pseudacorus* invasions in the southern hemisphere. If the ERH and EICA hold true for South African *I. pseudacorus* infestations, biological control could prove particularly valuable. As incipient invaders are often more vulnerable to biological control (Olckers et al., 2011), this may be an effective means to control *I. pseudacorus* before its invasions reach the severity observed elsewhere. However, the biological control of invasive species is a timely process, with results often taking years to materialize. As such, it may be of value to implement mechanical control programmes against *I. pseudacorus* until such a time that an appropriate biological control programme is developed and initiated, as has been the case for *H. verticillata* in South Africa (Coetzee et al., 2011b).

With sexual reproduction being dominant amongst *I. pseudacorus* populations in the United States, recommendations made for the management of such populations include: i) the prevention of seed development and maturation through changes in the timing of mechanical or chemical control; and ii) the development of a biological control agent from a guild capable of reducing seed production (Gaskin et al., 2016). As South African *I. pseudacorus* populations also employ sexual reproductive strategies to a large degree, these recommendations ought to be considered in South Africa as well as the United States, with the addition of a third recommendation: to increase public awareness around *I. pseudacorus* invasions and the negative environmental and economic effects thereof.

While it may not be feasible to manually remove large stands of *I. pseudacorus* in the country's more severe infestations, reducing seed production by removing flowers and seed capsules may aid in reducing seed dispersal, limiting the founding of novel populations. Considering the enhanced sexual reproductive output of invasive *I. pseudacorus* populations, reducing seed production is of great importance in managing and controlling infestations. If mechanical control of *I. pseudacorus* is implemented, it would be beneficial to remove plants prior to the seeding season. As support for gene-flow between populations has been found, it may prove most effective to remove plants before they start to flower.

As many of South Africa's *I. pseudacorus* infestations are 'artificial', controlling infestations chemically may be appropriate at certain sites. For example, while the risks of herbicide application in a natural or protected landscape may outweigh the benefits of controlling an invasion (Mikulyuk et al., 2020), the same will not necessarily hold true for an invasion in a modified landscape, such as a golf course. However, harmful knock-on effects may occur if the herbicide enters the water system and is carried outside of the 'artificial' environment.

While the use of mechanical or chemical control may provide some control of *I. pseudacorus* infestations for the time-being, these methods often prove costly (Maluleke et al., 2021), and their sustainability has been questioned (van Wilgen et al., 2001). For example, while an estimated ZAR 1,720,000 was spent on the management of *S. platyphylla* infestations in South

Africa between 2010 and 2019, the weed continues to spread at an exponential rate (Ndlovu et al., 2020). On the other hand, while the biological control of *I. pseudacorus* would likely prove more cost-effective and sustainable in the long run, waiting until such a time that a biological control programme is developed and initiated may allow for *I. pseudacorus* infestations to intensify. As such, an adaptive and integrated control programme may prove most effective in managing South Africa's *I. pseudacorus* invasion in the long term, whereby the relative application of mechanical and chemical control is dependent on the invaded system (van Wilgen et al., 2001), and the programme is revised when a suitable biological control programme has been developed.

The development of a biological control programme against *I. pseudacorus* has been initiated, with a flea beetle, *Aphthona nonstriata* Goeze (Coleoptera: Chrysomelidae), a seed weevil, *Mononychus punctumalbum* Herbst (Coleoptera: Curculionidae), and a sawfly, *Rhadinoceraea micans* Klug (Hymenoptera: Tenthredinidae), identified as prospective biological control agents (Minuti et al., 2021). The flea beetle, *A. nonstriata* is currently undergoing host-specificity testing at Rhodes University's Centre for Biological Control (CBC), while the remaining prospective candidates are awaiting importation (Minuti et al., 2021). The candidate agents attack different structures of the plant (Figure 4.3), and so their relative success in controlling *I. pseudacorus* will depend on the relative importance of each structure to the species' invasion. As the weevil, *M. punctumalbum*, attacks seed capsules, it should reduce the sexual reproductive output of *I. pseudacorus* populations. With the enhanced reproductive output observed in South African *I. pseudacorus* populations, *M. punctumalbum* may prove of great value in the effort to reduce the spread of *I. pseudacorus* in South Africa.

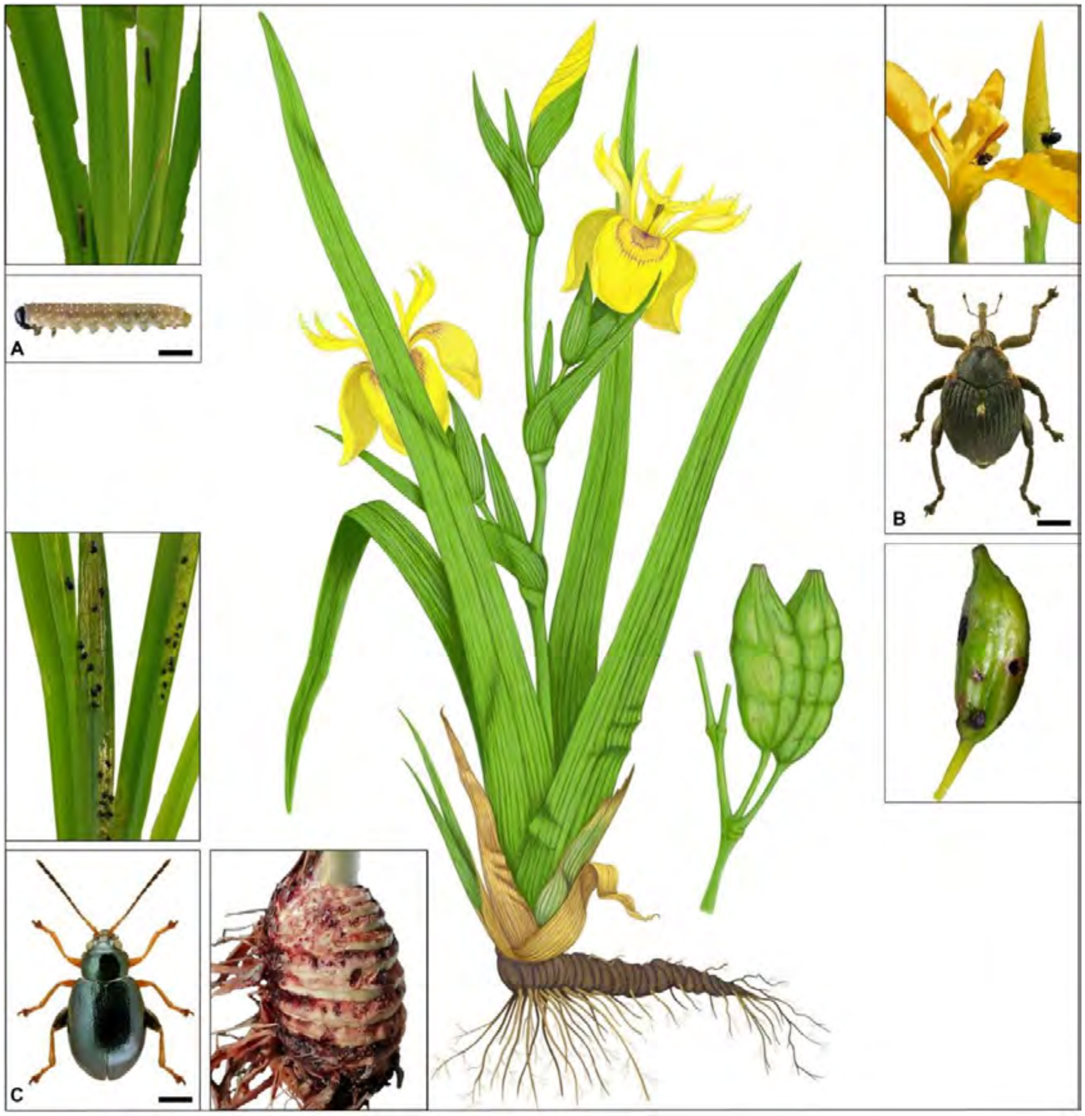


Figure 4.3: Schematic representation of *I. pseudacorus* and the damage inflicted by three prospective biological control agents, a) *Rhadinoceraea micans*, b) *Mononychus punctumalbum*, and c) *Aphthona nonstriata* (Source: Minuti et al., 2021)

The prospective biological control agents were identified as specialist natural enemies through field surveys in the native range of *I. pseudacorus*. As *I. pseudacorus* has a wide native distribution in Europe and western Asia, it would not have been feasible to sample throughout the native range, and so an eco-climate approach was followed (Wapshere, 1985; Minuti et al., 2021). This approach is useful in that prospective biological control agents will be sourced from climatically suitable regions to those in which they will be introduced.

With the identification of source populations through molecular techniques, the eco-climate approach could be augmented with the identification and sourcing of biological control agents which are both climate- and host-adapted (Paterson et al., 2014). By including a range of indigenous *I. pseudacorus* samples in further genetic analyses, it may be possible to identify source populations of the weed, to which populations of potential biological control agents may or may not demonstrate local adaptation (Paterson et al., 2014). While molecular matching of native and invasive *I. pseudacorus* populations has yet to occur, *A. nonstriata* populations are being maintained on South African *I. pseudacorus* plant material, and so maladaptation to the South African *I. pseudacorus* genotype is unlikely. As *A. nonstriata* does not attack the sexual reproductive structures of *I. pseudacorus*, it may be useful to investigate the suitability of *M. punctumalbum* instead of, or in tandem with, *A. nonstriata* to control invasive *I. pseudacorus* populations.

Finally, as *I. pseudacorus* spreads largely through the sharing of seed or vegetative fragments amongst horticulturalists, landowners, and gardening enthusiasts, human activity is largely responsible for the invasion of *I. pseudacorus* in South Africa. However, with effective management strategies and increased public awareness and support, the role of human activity in species invasions may become less ominous and increasingly constructive (Vanderhoeven et al., 2011). The ongoing social media campaign encouraging the public to report *I. pseudacorus* infestations to the Centre for Biological Control has been successful, with much public interest expressed on the CBC's various social media platforms. This may be promising for further public engagement involving the management and control of *I. pseudacorus* invasions in South Africa. As such, ways in which to expand the reach of public awareness campaigns regarding *I. pseudacorus* invasions should be explored.

4.3 Global implications

These results contribute to the global south initiative to understand and control *I. pseudacorus* invasions across the southern hemisphere. As *I. pseudacorus* has a broad distribution and occurs across a range of environmental conditions, it is likely that the autecology of *I. pseudacorus* populations will differ across the species' invasive range. As such, these findings and their implications cannot necessarily be inferred outside of a South African context. Through the collaborative efforts of researchers in South Africa, Argentina, and New Zealand, similar studies are being conducted across the southern hemisphere, aiming to provide further insight into the invasion autecology of *I. pseudacorus* in the global south.

As the development of a biological control programme to manage *I. pseudacorus* invasions forms part of this collaborative effort, it is important to understand the invasion biology and ecology of invasive *I. pseudacorus* populations across their invasive range. I recommend that the development of an appropriate biological control programme for *I. pseudacorus* in South Africa should include a biological control agent from a guild capable of reducing seed production, development, and/or maturation. While this was also suggested by Gaskin et al. (2016) for the management of *I. pseudacorus* populations in the United States, this may not be appropriate across the invasive range of *I. pseudacorus,* particularly in the southern hemisphere where flowering is asynchronous with the emergence of the seed-feeding weevil.

4.4 Conclusions

This study provides insight into the invasion autecology of *I. pseudacorus* populations in South Africa, and contributes to the growing body of research surrounding *I. pseudacorus* invasions worldwide. Specifically, the results of this study contribute to the global south initiative aimed to manage and control *I. pseudacorus* invasions across the southern hemisphere.

By confirming the distribution of *I. pseudacorus* populations in South Africa and determining the relative abundance of individuals in each population, high- and low-risk systems can be identified, and appropriate management strategies for each site can be developed. The relatively high employment of sexual reproductive strategies amongst South African *I. pseudacorus* populations provides valuable information regarding the species' reproductive and dispersive abilities, allowing for management and control of *I. pseudacorus* to proceed in an informed and effective manner.

Iris pseudacorus is still in the 'lag' phase of its invasion in South Africa. However, these results and the severity of infestations in Argentina, for example, caution that South Africa is not immune to further invasion by *I. pseudacorus*. As such, it is important that current infestations be managed and controlled effectively, while efforts are made to prevent further invasion.

References

Abbot, P. 2001. Individual and population variation in invertebrates revealed by Inter-simple sequence repeats (ISSRs). *Journal of Insect Science* 1, 8.

Abbott, R.J. 1992. Plant invasions, interspecific hybridization and the evolution of new plant taxa. *Trends in Ecology & Evolution* 7(12): 401-405.

Alford, R.A., Wilbur, H.M. 1985. Priority effects in experimental pond communities: competition between Bufo and Rana. *Ecology* 66, 1097-1105.

Armas, C., Ordiales, R., Pugnaire, F.I. 2004. Measuring plant interactions: A new comparative index. *Ecology* 85(10), 2682-2686.

Arrigo, N., Holderegger, R., Alvarez, N. 2012. Automated scoring of AFLPs using RawGeno v2.0, a free R CRAN library. In: Pompanon, F., Bonin, A. (eds) Data Production and Analysis in Population Genomics. Methods in Molecular Biology (Methods and Protocols), vol 888. Humana Press, Totowa, NJ.

Arrigo, N., Tuszynski, J.W., Ehrich, D., Gerdes, T., Alvarez, N. 2009. Evaluating the impact of scoring parameters on the structure of intra-specific genetic variation using RawGeno, an R package for automating AFLP scoring. *BMC Bioinformatics* 10, 33.

Baker, H.G. 1955. Self-compatibility and establishment after ‘long-distance’ dispersal. *Evolution* 9(3), 347-349.

Baker, R.H.A., Sansford, C.E., Jarvis, C.H., Cannon, R.J.C., MacLeod, A., Walters, K.F.A. 2000. The role of climatic mapping in predicting the potential geographical distribution of non-indigenous pests under current and future climates. *Agriculture, Ecosystems & Environment* 82(1-3), 57-71.

Balestri, E., Menicagli, V., Lardicci, C. 2021. Managing biotic interactions during early seagrass life stages to improve seed-based restoration. *Journal of Applied Ecology* 00, 1-10.

Barker, N.P., Paterson, I., Howis, S. 2015. “Barcoding” and ISSR data illuminate a problematic infraspecific taxonomy for *Chrysanthemoides monilifera* (Calenduleae; Asteraceae): Lessons for biocontrol of a noxious weed. *Biochemical Systematics and Ecology*, 61: 541-551.

Barrett, S.C.H., Colautti, R.I., Eckert, C.G. 2008. Plant reproductive systems and evolution during biological invasion. *Molecular Ecology* 17, 373-383.

Bauer, J.T. 2012. Invasive species: "back-seat drivers" of ecosystem change? *Biological Invasions* 14, 1295-1304.

Benjamin, L.R., Hardwick, R.C. 1986. Sources of variation and measures of variability in even-aged stands of plants. *Annals of Botany* 58(6), 757-778.

Blackburn, T.M., Pyšek, P., Bacher, S., Carlton, J.T., Duncan, R.P., Jarošik, J., Wilson, J.R.U., Richardson, D.M. 2011. A proposed unified framework for biological invasions. *Trends in Ecology and Evolution* 26(7), 333-339.

Blair, A.C., Wolfe, L.M. 2004. The evolution of an invasive plant: an experimental study with *Silene latifolia*. *Ecology* 85(11), 3035-3042.

Blossey, B., Nötzold, R. 1995. Evolution of increased competitive ability in invasive nonindigenous plants: a hypothesis. *Journal of Ecology* 83, 887-889.

Bock, D.G., Caseys, C. Cousens, R.D., Hahn, M.A., Heredia, S.M., Hübner, S., Turner, K.G., Whitney, K.D., Rieseberg, L.H. 2014. What we still don't know about invasion genetics. *Molecular Ecology* 24(9): 2277-2297.

Bright, C. 1999. Invasive Species: Pathogens of Globalization. *Foreign Policy* 116, 50-64.

Buckley, Y.M., Downey, P., Fowler, S.V., Hill, R., Memmot, J., Norambuena, H., Pitcairn, M., Shaw, R., Sheppard, A.W., Winks, C., Wittenberg, R., Rees, M. 2003. Are invasives bigger? A global study of seed size variation in two invasive shrubs. *Ecology* 84(6), 1434-1440.

Burke, M.J.W., Grime, J.P. 1996. An experimental study of plant community invasibility. *Ecology* 77(3), 776-690.

Burns, J.H. 2004. A comparison of invasive and non-invasive dayflowers (Commelinaceae) across experimental nutrient and water gradients. *Diversity and Distributions* 10(5-6), 387-197.

Callaway, R.M., Ridenour, W.M. 2004. Novel weapons: invasive success and the evolution of increased competitive ability. *Frontiers in Ecology and the Environment* 2(8), 436-443.

Coetzee, J.A., Hill, M.P. 2012. The role of eutrophication in the biological control of water hyacinth, *Eichhornia crassipes*, in South Africa. *BioControl* 57(2), 247-261.

Coetzee, J.A., Bownes, A., Martin, G.D. 2011b. Prospects for the Biological control of submerged macrophyes in South Africa. *African Entomology* 19(2), 469-487.

Coetzee, J.A., Hill, M.P., Byrne, M.J., Bownes, A. 2011a. A review of the biological control programmes on *Eichhornia crassipes* (C.Mart.) Solms (Pontederiaceae), *Salvinia molesta* D.S.Mitch. (Salviniaceae), *Pistia stratiotes* L. (Araceae), *Myriophyllum aquaticum* (Vell.) Verdc. (Haloragaceae) and *Azolla filiculoides* Lam. (Azollaceae) in South Africa. *African Entomology* 19(2), 451-468.

Colautti, R.I., Grigorovich, I.A., MacIsaac, H.J. 2006. Propagule pressure: A null model for biological invasions. *Biological Invasions* 8, 1023-1037.

Coops, H., van der Velde, G. 1995. Seed dispersal, germination and seedling growth of six helophyte species in relation to water-level zonation. *Freshwater Biology* 34, 13-20.

Correia, M., Montesinos, D., French, K., Rodríguez-Echeverría, S. 2016. Evidence for enemy release and increased seed production and size for two invasive Australian acacias. *Journal of Ecology* 104(5), 1391-1399.

Cory, J.S., Myers, J.H. 2004. Adaptation in an insect host-plant pathogen interaction. *Ecology Letters* 7, 632-639.

Crawley, M.J. 1987. What makes a community invisible? *Colonization, Succession and Stability* (eds A. J. Gray, M.J. Crawley & P.J. Edwards), 429-453. Blackwell Scientific Publications, Oxford.

Crawley, M.J. 1990. The population dynamics of plants. *Philosophical Transactions: Biological Sciences* 330(1257), 125-140.

Crooks, J.A. 2002. Characterizing ecosystem-level consequences of biological invasions: the role of ecosystem engineers. *Oikos* 97, 153-166.

Daehler, C.C. 2003. Performance comparisons of co-occurring native and alien invasive plants: Implications for conservation and restoration. *Annual Review of Ecology, Evolution, and Systematics* 34, 183-211.

Davis, A.J., Jenkinson, L.S., Lawton, J.H., Shorrocks, B., Wood, S. 1998a. Making mistakes when predicting shifts in species range in response to global warming. *Nature* 391, 783-386.

Davis, A.J., Lawton, J.L., Shorrocks, B., Jenkinson, L.S. 1998b. Individualistic species responses invalidate simple physiological models of community dynamics under global environmental change. *Journal of Animal Ecology* 67, 600-612.

Davis, E., Caffrey, J.M., Coughlan, N.E., Dick, J.T.A., Lucy, F.E. 2018. Communications, outreach and citizen science: spreading the word about invasive alien species. *Management of Biological Invasions* 9(4), 515-525.

Davis, M.A. 2009. *The Invasion Process*. In: Invasion Biology. Oxford University Press, Oxford, 13-96.

Davis, M.A., Grime, J.P., Thompson, K. 2000. Fluctuating resources in plant communities: a general theory of invasibility. *Journal of Ecology* 88(3), 528-534.

Daws, M.I., Hall, J., Flynn, S., Pritchard, H.W. 2007. Do invasives have bigger seeds? Evidence from intra- and inter-specific comparisons. *South African Journal of Botany* 73, 138-143.

Department of Environmental Affairs. 2016. Alien and Invasive Species Lists, 2016 [Online] Available: https://www.environment.co.za/wp-content/uploads/2017/03/nemba10of2004_alienandinvasive_specieslists2016.pdf [Accessed 22/07/2019].

Dyer, A.F. 1976. The karyotype of *Iris pseudacorus* L. *Transactions of the Botanical Society of Edinburgh* 42(4), 421-429.

Ellenberg, H. (1979) Zeigerwerte von Gefässpflanzen Mitteleuropas. *Scripta Geobotanica* 9, 1-122.

Ellstrand, N.C., Schierenbeck, K.A. 2000. Hybridization as a stimulus for the evolution of invasiveness in plants? *Proceedings of the National Academy of Sciences of the United States of America* 97(13): 7043-7050.

Engin, A., Kandemir, N., Şenel, G., Ǒzkan, M. 1998. An autecological study on *Iris pseudacorus* L. (Iridaceae). *Turkish Journal of Botany* 22, 335-340.

Evanno, G., Regnaut, S., Goudet, J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* 14, 2611-2620.

Falush, D., Stephens, M., Pritchard, J.K. 2003. Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. *Genetics* 164(4), 1567-1587.

Falush, D., Stephens, M., Pritchard, J.K. 2007. Inference of population structure using multilocus genotype data: Dominant markers and null alleles. *Molecular Ecology Notes* 7(4), 574-578.

Fritz, R.S., Moulia, C., Newcombe, G. 1999. Resistance of hybrid plants and animals to herbivores, pathogens, and parasites. *Annual Review of Ecology and Systematics* 30, 565-591.

Fukami, T. 2015. Historical contingency in community assembly: Integrating niches, species pools, and priority effects. *Annual Review of Ecology, Evolution and Systematics* 46, 1-23.

García-Ramos, G., Rodríguez, D. 2002. Evolutionary speed of species invasions. *Evolution* 56(4), 661-668.

Gaskin, J.F., Pokorny, M.L., Mangold, J.M. 2016. An unusual case of seed dispersal in an invasive aquatic; yellow flag iris (*Iris pseudacorus*). *Biological Invasions* 18, 2067-2075.

Gaskin, J.F., Bon, M.C., Cock, M.J.W., Cristofaro, M., De Biase, A., De Clerck-Floate, R., Ellison. C.A., Hinz, H.L., Hufbauer, R.A., Julien, M.H., Sforza, R. 2011. Applying molecular-based approaches to classical biological control of weeds. *Biological Control* 58, 1-21.

Geange, S.W., Stier, A.C. 2010. Priority effects and habitat complexity affect the strength of competition. *Oecologia* 163, 111-118.

Gervazoni, P., Sosa, A., Franceschini, C., Coetzee, J., Faltlhauser, A., Fuentes-Rodriguez, D., Martínez, A., Hill, M. 2020. The alien invasive yellow flag (*Iris pseudacorus* L.) in Argentinian wetlands: assessing geographical distribution through different data sources. *Biological Invasions* 22, 3183-3193.

Gilbert, K.J., Andrew, R.L., Bock, D.G., Franklin, M.T., Kane, N.C., Moore, J.S., Moyers, B.T., Renaut, S., Rennison, D.J., Veen, T., Vines, T.H. 2012. Recommendations for utilizing and reporting population genetic analyses: The reproducibility of genetic clustering using the program structure. *Molecular Ecology* 21, 4925-4930.

Gillard, M.B., Castillo, J.M., Mesgaran, M.B., Futrell, C.J., Grewell, B.J. 2021. High aqueous salinity does not preclude germination of invasive *Iris pseudacorus* from estuarine populations. *Ecosphere* 12(5), e03486.

Gómez-Baggethun, E., Ruiz-Pérez, M. 2011. Economic valuation and the commodification of ecosystem services. *Progress in Physical Geography* 35(5), 613-628.

Goolsby, J.A., van Klinken, R.D., Palmer, W.A. 2006. Maximising the contribution of native-range studies towards the identification and prioritisation of weed biocontrol agents. *Australian Journal of Entomology* 45, 276-286.

Grewell, B.J., Gallego-Tévar, B., Gillard, M.B., Futrell, C.J., Reicholf, R., Castillo, J.M. 2021. Salinity and inundation effects on *Iris pseudacorus*: implications for tidal wetland invasion with sea level rise. *Plant and Soil* 466, 275-291.

Hamada, H., Petrino, M.G., Kakunaga, T. 1982. A novel repeated element with Z-DNA-forming potential is widely found in evolutionarily diverse eukaryotic genomes. *Proceedings of the National Academy of Sciences* 76, 6465-6469.

Hammer, Ø., Harper, D.A.T., Ryan, P.D. 2001. PAST: Paleontological statistics software package for education and data analysis. Palaeontologia Electronica 4, 1–9.

Harvey, J.A., Bukovinszky, T. van der Putten, W.H. 2010. Interactions between invasive plants and insect herbivores: A plea for a multitrophic perspective. *Biological Conservation* 143, 2251-2259.

Hierro, J.L., Villarreal, D., Eren, Ö., Graham, J.M., Callaway, R.M. 2006. Disturbance facilitates invasion: The effects are stronger abroad than at home. *The American Naturalist* 168(2), 144-156.

Hill, M.P. 2003. The impact and control of alien aquatic vegetation in South African aquatic ecosystems. *African Journal of Aquatic Science* 28, 19-24.

Hill, M.P., Coetzee, J. 2017. The biological control of aquatic weeds in South Africa: current status and future challenges. *Bothalia: African Biodiversity & Conservation* 47(2), 1-12.

Hill, M.P., Coetzee, J.A., Martin, G.D., Smith, R., Strange, E.F. 2020. *Invasive alien aquatic plants in South African freshwater ecosystems*. In: van Wilgen, B., Measey, J., Richardson, D., Wilson, J., Zengeya, T. (eds) Biological Invasions in South Africa. Invading Nature – Springer Series in Invasion Ecology, vol 14. Springer, Cham., 97-114.

Hinz, H.L., Winston, R.L., Schwarzländer, M. 2019. How safe is weed biological control? A global review of direct nontarget attack. *The Quarterly Review of Biology* 94, 1-27.

Hodkinson, D., Thompson, K. 1997. Plant dispersal: the role of man. *Journal of Applied Ecology* 34, 1484-1496.

Holland, B.R., Clarke, A.C., Meudt, H.M. 2008. Optimizing automated AFLP scoring parameters to improve phylogenetic resolution. *Systematic Biology* 57, 347-366.

Hubisz, M.J., Falush, D., Stephens, M., Pritchard, J.K. 2009. Inferring weak population structure with the assistance of sample group information. *Molecular Ecology Resources* 9(5), 1322-1332.

Hui, C., Richardson, D.M. 2017. *Invasion Dynamics.* Oxford University Press, Oxford. Pp.

Huson, D.H., Bryant, D. 2006. Application of phylogenetic networks in evolutionary studies. *Molecular Biology and Evolution* 23(2), 254-267.

Huxel, G.R. 1999. Rapid displacement of native species by invasive species: effects of hybridization. *Biological Conservation* 89(2), 143-152.

Jaca, T.P. 2013. *Iris pseudacorus* L.: An ornamental aquatic with invasive potential in South Africa. *South African Journal of Botany* 86, 174.

Jaca, T.P., Mkhize, V. 2015. Distribution of *Iris pseudacorus* (Linnaeus, 1753) in South Africa. *BioInvasions Records* 4, 249-253.

Jacobs, J., Graves, M., Mangold, J. 2010. Ecology and management of Yellowflag Iris (*Iris pseudacorus* L.) Invasive Species Technical Note no. MT-28, National Resources Conservation Service, United States Department of Agriculture, Montana.

Johansson, M.E., Nilsson, C. 1993. Hydrochory, population dynamics and distribution of the clonal aquatic plant *Ranunculus lingua*. *Journal of Ecology* 81, 81-91.

Joshi, J., Vrieling, K. 2005. The enemy release and EICA hypothesis revisited: Incorporating the fundamental difference between specialist and generalist herbivores. *Ecology Letters* 8(7), 704-714.

Keane, R.M., Crawley, M.J. 2002. Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology & Evolution* 17(4), 164-170.

Kettenring, K.M., Adams, C.R. 2011. Lessons learned from invasive plant control experiments: a systematic review and meta-analysis. *Journal of Applied Ecology* 48(4), 970-979.

Kopelman, N.M., Mayzel, J., Jakobsson, M., Rosenberg, N.A., Mayrose, I. 2015. Clumpak: A program for identifying clustering modes and packaging population structure inferences across K. *Molecular Ecology Resources* 15, 1179-1191.

Kühn, I., Wolf, J., Schneider, A. 2017. Is there an urban effect in alien plant invasions? *Biological Invasions* 19, 3505-3513.

Kwong, R.M., Sagliocco, J.L., Harms, N.E., Butler, K.L., Martin, G.D., Green, P.T. 2019. Could enemy release explain the invasion success of *Sagittaria platyphylla* in Australia and South Africa? *Aquatic Botany* 153, 67-72.

Lamote, V., Roldán-Ruiz, I., Coart, E., De Loose, M., Van Bockstaele, E. 2002. A study of genetic variation in *Iris pseudacorus* populations using amplified fragment length polymorphisms (AFLPs). *Aquatic Botany* 73, 19-31.

Lawton, J.L. 2000. Concluding remarks: a review of some open questions. Ecological consequences of heterogeneity. (ed. Hutchings, M.J., Stewart, J. and Stewart, A.J.A.), 401-424. Cambridge University Press, Cambridge.

Le Roux, J., Wieczorek, A.M. 2009. Molecular systematics and population genetics of biological invasions: towards a better understanding of invasive species management. *Annuls of Applied Biology*, 154: 1-17.

Lear, L., Hesse, E., Shea, K., Buckling, A. 2020. Disentangling the mechanisms underpinning disturbance-mediated invasion. *Proceedings of the Royal Society B* 287(1919), 20192415.

Lee, C.E. 2002. Evolutionary genetics of invasive species. *Trends in Ecology & Evolution* 17(8): 386-391.

Leng, X., Musters, C.J.M., de Snoo, G.R. 2009. Restoration of plant diversity on ditch banks: Seed and site limitation in response to agri-environment schemes. *Biological Conservation* 142(7), 1340-1349.

Levine, J.M., D'Antonio, C.M. 1999. Elton revisited: A review of evidence linking diversity and invasibility. *Oikos* 87, 15-26.

Li, Y.L., Liu, J.X. 2018. StructureSelector: A web-based software to select and visualize the optimal number of clusters using multiple methods. *Molecular Ecology Resources* 18, 176-177.

Lockwood, J.L., Cassey, P., Blackburn, T.M. The more you introduce the more you get: the role of colonization pressure and propagule pressure in invasion ecology. *Diversity and Distributions* 15(5), 904-910.

Lowe, S., Browne, M., Boudjelas, S., De Poorter, M. 2002. *100 of the World's Worst Invasive Alien Species: A Selection from the Global Invasive Species Database*. Published by the Invasive Species Specialist Group (ISSG) a specialist group of the Species Survival Commission (SSC) of the World Conservation Union (IUCN), 12pp.

MacDougall, A.S., Turkington, R. 2005. Are invasive species the drivers or passengers of change in degraded ecosystems? *Ecology* 86, 42-55.

Mack, R.N., Simberloff, D., Lonsdale, W.M., Evans, H., Clout, M., F.A. Bazzaz. 2000. Biotic invasions: causes, epidemiology, global consequences, and control. *Ecological Applications* 10(3), 689-710.

Maki, K., Galatowitsch, S. 2004. Movement of invasive aquatic plants into Minnesota (USA) through horticultural trade. *Biological Conservation* 118(3), 389-396.

Maluleke, M., Fraser, G.C.G., Hill, M.P. 2021. Economic evaluation of chemical and biological control of four aquatic weeds in South Africa. *Biocontrol Science and Technology* 31(9), 896-911.

Martin, G.D., Coetzee, J.A. 2011. Pet stores, aquarists and the internet trade as modes of introduction and spread of invasive macrophytes in South Africa. *Water SA* 37(3), 371-380.

McConnachie, A.J., de Wit, M.P., Hill, M.P., Byrne, M.J. 2003. Economic evaluation of the successful biological control of *Azolla filiculoides* in South Africa. *Biological Control* 28, 25-32.

McLean, P., Gallien, L., Wilson, J.R.U., Gaertner, M., Richardson, D.M. 2017. Small urban centres as launching sites for plant invasions in natural areas: insights from South Africa. *Biological Invasions* 19, 3541-3555.

Mikulyuk, A., Kujawa, E., Nault, M.E., Van Egeren, S., Wagner, K.I., Barton, M., Hauxwell, J., Vander Zanden, M.J. 2020. Is the cure worse than the disease? Comparing the ecological effects of an invasive aquatic plant and the herbicide treatments used to control it. *Facets* 5, 353-366.

Minuti, G., Stiers, I., Coetzee, J.A. 2022. Climatic suitability and compatibility of the invasive *Iris pseudacorus* L. (Iridaceae) in the Southern Hemisphere: Considerations for biocontrol. *Biological Control* 169, 104886.

Minuti, G., Coetzee, J.A., Ngxande-Koza, S., Hill, M.P., Stiers, I. 2021. Prospects for the biological control of *Iris pseudacorus* L. (Iridaceae). *Biocontrol Science and Technology* 31(3), 314-335.

Mopper, S., Strauss, S.Y. 1998. *Genetic structure and local adaptation in natural insect populations: effects of ecology, life history, and behaviour.* Springer Science & Business Media.

Mopper, S., Wiens, K.C., Goranova, G.A. 2016. Competition, salinity, and clonal growth in native and introduced irises. *American Journal of Botany* 103(9), 1575-1581.

Morgan, V.H. 2010. *Iris pseudacorus*. USGS Nonindigenous Aquatic Species Database, Gainesville, FL. http://nas.er.usgs. gov/queries/FactSheet.aspx?SpeciesID=1115.

Morgan, V.H., Berent, L., Fusaro, A. 2020. *Iris pseudacorus* L.: U.S. Geological Survey, Nonindigenous Aquatic Species Database, Gainesville, FL, https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=1115, Revised: 8/8/2018, Accessed: 4/20/2020

Muhlfeld, C.C., Kalinowski, S.T., McMahon, T.E., Taper, M.L., Painter, S., Leary, R.F., Allendorf, F.W. 2009. Hybridization rapidly reduces fitness of a native trout in the wild. *Biology Letters* 5, 328-331.

Mullin, B.H., Anderson, L.W.J., Ditomaso, J.M., Eplee, R.E., Getsinger, K.D. 2000. Invasive plant species. *Council for Agricultural Science and Technology* 13, 1-18.

Ndlovu, M.S., Coetzee, J.A., Nxumalo, M.M., Lalla, R., Shabalala, N., Martin, G.D. 2020. The establishment and rapid spread of *Sagittaria platyphylla* in South Africa. *Water* 12(5), 1472.

Nei, M. 1975. *Molecular population genetics and evolution*. North-Holland Publishing Company, Amsterdam.

Núñez-González, N., Rodríguez, J., González, L. 2021. Managing the invasive plant *Carpobrotus edulis*: is mechanical control or specialized natural enemy more effective? *Journal of Environmental Management* 298, 113554.

Olden, J.D., Poff, N.L., Douglas, M.R., Douglas, M.E., Fausch, K.D. 2004. Ecological and evolutionary consequences of biotic homogenization. *Trends in Ecology and Evolution* 19, 18-24.

Ouborg, N.J., Piquot, Y., Van Groenendael, J.M. 2008. Population genetics, molecular markers and the study of dispersal in plants. *Journal of Ecology* 87(4), 551-568.

Paterson, I.D., Zachariades, C. 2013. ISSRs indicate that *Chromolaena odorata* invading southern Africa originates in Jamaica or Cuba. *Biological Control* 66, 132-139.

Paterson, I., Downie, D.A., Hill, M.P. 2009. Using molecular methods to determine the origin of weed populations of *Pereskia aculeata* in South Africa and its relevance to biological control. *Biological Control*, 48: 84-91.

Paterson, I.D., Hill, M.P., Downie, D.A. 2012. The effect of host plant intraspecific genetic variation on the fitness of a monophagous biological control agent. *Biocontrol Science and Technology* 22(5), 513-525.

Paterson, I.D., Vitorino, M.D., de Cristo, S.C., Martin, G.D., Hill, M.P. 2014. Prioritisation of potential agents for the biological control of the invasive alien weed, *Pereskia aculeata* (Cactaceae), in South Africa. *Biocontrol Science and Technology* 24(4), 407-425.

Pearson, D.E., Ortega, Y.K., Eren, Ö., Hierro, J. 2018. Community assembly theory as a framework for biological invasions. *Trends in Ecology & Evolution* 33(5), 313-325.

Pejchar, L., Mooney, H.A. 2009. Invasive species, ecosystem services and human well-being. *Trends in Ecology & Evolution* 24(9), 497-504.

Peterson, G., Allen, C.R., Holling, C.S. 1998. Ecological resilience, biodiversity, and scale. *Ecosystems* 1, 6-18.

Pimentel, D., Zuniga, R., Morrison, D. 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics* 52(3), 273-288.

Posada, D., Crandall, K.A. 2001. Selecting the best-fit model of nucleotide substitution. *Systematic Biology* 50(4), 580-601.

Powell, W., Machray, G.C., Provan, J. 1996. Polymorphism revealed by simple sequence repeats. *Trends in Plant Science* 1(7), 215-222.

Pritchard, J.K., Stephens, M., Donnelly, P. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155(2), 945-959.

Puechmaille, S.J. 2016. The program STRUCTURE does not reliably recover the correct population structure when sampling is uneven: subsampling and new estimators alleviate the problem. *Molecular Ecology Resources* 16(3), 608-627.

Pyšek, P., Jarošik, V., Chytrý, M., Danihelka, J., Kühn, I., Pergl, J., Tichý, L., Biesmeijer, J.C., Ellis, W.N., Kunin, W.E., Settele, J. 2011. Successful invaders co-opt pollinators of native flora and accumulate insect pollinators with increasing residence time. *Ecological Monographs* 81(2), 277-193.

Qi, Y., Wei, W., Chen, C., Chen, L. 2019. Plant root-shoot biomass allocation over diverse biomes: A global synthesis. *Global Ecology and Conservation* 18, e00606.

Reaser, J.K., Burgiel, S.W., Kirkey, J., Brandley, K.A., Veatch, S.D., Burgos-Rodríguez, J. 2020. The early detection of and rapid response (EDRR) to invasive species: a conceptual framework and federal capacities assessment. *Biological Invasions* 22, 1-19.

Reichard, S.H., White, P. 2001. Horticulture as a pathway of invasive plant introductions in the United States. *BioScience* 51(2), 103-113.

Reid, M.K., Naidu, P., Paterson, I.D., Mangan, R., Coetzee, J.A. 2021. Population genetics of invasive and native *Nymphaea mexicana* Zuccarini: Taking the first steps to initiate a biological control programme in South Africa. *Aquatic Botany* 171, 103372.

Rosbakh, S., Phartyal, S.S., Poschlod, P. 2020. Seed germination traits shape community assembly along a hydroperiod gradient. *Annals of Botany* 125, 67-78.

Rouget, M., Robertson, M.P., Wilson, J.R.U., Hui, C., Essl, F., Renteria, J.L., Richardson, D.M. 2015. Invasion debt – quantifying future biological invasions. *Diversity and Distributions* 22, 445-456.

Roy, J. 1990. In search of the characteristics of plant invaders, in: di Castri, F., Hansen, A.J., Debussche, M. (Eds.), *Biological Invasions in Europe and the Mediterranean Basin.* Monographiae Biologicae, vol 65. Springer, Dordrecht.

Sakai, A.K., Allendorf, F.W., Holt, J.S., Lodge, D.M., Molofsky, J., With, K.A., Baughman, S., Cabin, R.J., Cohen, J.E., Ellstrand, N.C., McCauley, D.E., O'Neil, P., Parker, I.M., Thompson, J.N., Weller, S.G. 2001. The population biology of invasive species. *Annal Review of Ecology and Systematics* 32, 305-332.

Scheffer, M., van Nes, E.H. 2007. *Shallow lakes theory revisited: various alternative regimes driven by climate, nutrients, depth and lake size.* In: Gulati, R.D., Lammens, E., De Pauw, N., Van Donk, E. (eds) Shallow Lakes in a Changing World. Developments in Hydrobiology, vol 196. Springer, Dordrecht.

Scott, J.K. 1996. Population ecology of Chrysanthemoides monilifera in South Africa: Implications for its control in Australia. *Journal of Applied Ecology* 33(6), 1496-1508.

Smith, V.H., Tilman, G.D., Nekola, J.C. 1999. Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. *Environmental Pollution* 100(1-3), 179-196.

Sunny, A., Diwakar, S., Sharma, G.P. Native insects and invasive plant encounters. *Arthropod-Plant Interactions* 9, 323-331.

Sutherland, W.J. 1990. *Iris pseudacorus* L. *Journal of Ecology* 78(3), 833-848.

Sutton, G.F., Paterson, I.D., Paynter, Q. 2017. Genetic matching of invasive populations of the African tulip tree, *Spathodea campanulate* Beauv. (Bignoniaceae), to their native distribution: Maximising the likelihood of selecting host-compatible biological control agents. *Biological Control* 114, 167-175.

Thomas, L.K. 1980. The impacts of three exotic plant species on Potomac Island. Monograph Series 13. National Park Service, Washington, D.C.

Thompson, J.N., Weller, S.G. The population biology of invasive species. *Annual Review of Ecology and Systematics* 32: 305-332.

Shackleton, C.M., McGarry, D., Fourie, S., Gambiza, J., Shackleton, S.E., Fabricius, C. 2007. Assessing the effects of invasive alien species on rural livelihoods: Case examples and a framework from South Africa. *Human Ecology* 35: 113-127.

Silvertown, J. 2008. The evolutionary maintenance of sexual reproduction: Evidence from the ecological distribution of asexual reproduction in clonal plants. *International Journal of Plant Sciences* 169, 157-168.

Van Ginkel, C.E. 2011. Eutrophication: present reality and future challenges for South Africa. *Water SA* 37(5), 693-701.

Van Wilgen, B.W., De Lange, W.J. 2011. The costs and benefits of biological control of invasive alien plants in South Africa. *African Entomology* 19(2), 504-514.

Van Wilgen, B., Richardson, D., Higgins, S. 2001. Integrated control of invasive alien plants in terrestrial ecosystems. *Land Use and Water Resources Research* 5, 1-6.

Vanderhoeven, S., Piqueray, J., Halford, M., Nulens, G., Vincke, J., Mahy, G. 2011. Perception and understanding of invasive alien species issues by nature conservation and horticulture professionals in Belgium. *Environmental Management* 47, 425-442.

Vilà, M., Weiner, J. 2004. Are invasive plant species better competitors than native plant species? – evidence from pair-wise experiments. *Oikos* 105(2), 229-238.

Vilà, M., Weber, E., D'Antonio, C.M. 2000. Conservation implications of invasion by plant hybridization. *Biological Invasions* 2, 207-217.

Vilà, M., Espinar, J.L., Hejda, M., Hulme, P.E., Jarošik, V., Maron, J.L., Pergl, J., Schaffner, U., Sun, Y., Pyšek, P. 2011. Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems. *Ecology Letters* 14, 702-708.

Vitousek, P.M., D'Antonio, C.M., Loope, L.L., Rejmánek, M., Westbrooks, R. 1997. Introduced species: A significant component of human-caused global change. *New Zealand Journal of Ecology* 21: 1-16.

Wania, A., Kühn, I., Klotz, S. 2006. Plant richness patterns in agricultural and urban landscapes in Central Germany – spatial gradients of species richness. *Landscape and Urban Planning* 75(1-2), 97-110.

Wapshere, A.J. 1985. Effectiveness of biological control agents for weeds: Present quandaries. *Agriculture, Ecosystems & Environment* 13(3-4), 261-280.

Whitton, J., Sears, C.J., Baack, E.J., Otto, S.P. 2008. The dynamic nature of apomixis in the angiosperms. *International Journal of Pant Sciences* 169, 169-182.

Williamson, M. 1996. *Biological Invasions.* Chapman and Hall, London. Pp.

Wolfe, A.D., Xiang, Q., Kephart, S.R. 1998. Assessing hybridization in natural populations of *Penstemon* (Scrophulariaceae) using hypervariable intersimple sequence repeat (ISSR) bands. *Molecular Ecology* 7(9), 1107-1125.

Woodward, F.I. 1987. Climate and Plant Distribution. Cambridge University Press, Cambridge.

Yousefi, Z., Mohseni-Bandpei, A. 2010. Nitrogen and phosphorus removal from wastewater by subsurface wetlands planted with *Iris pseudacorus*. *Ecological Engineering* 36(6), 777-782.

Zalucki, M.P., Day, M.D., Playford, J. 2007. Will biological control of *Lantana camara* ever succeed? Patterns, processes & prospects. *Biological Control* 42, 251-261.

Zhang, S., Lamb, E.G. 2012. Plant competitive ability and the transitivity of competitive hierarchies change with plant age. *Plant Ecology* 213, 15-23.