An initial investigation into biological control options for *Schinus terebinthifolia* in South Africa

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Abstract

Schinus terebinthifolia Raddi (Anacardiaceae) (Brazilian pepper tree) is a native tree to subtropical South America that was introduced into South Africa as an ornamental plant. Globally, it is regarded as one of the world's worst invasive trees. In South Africa, this aggressive pioneer species is becoming increasingly problematic and is being considered as a target for biological control.

In South Africa the tree has acquired a native seed-feeding wasp, *Megastigmus transvaalensis* Hussey (Hymenoptera: Torymidae). The wasp's native hosts are indigenous *Rhus* species (Anacardiaceae), but it has expanded its host range to form a new association with both *S. terebinthifolia* and its close relative *S. molle* L. (Anacardiaceae). In order to quantify the seed predation by *M. transvaalensis* on *S. terebinthifolia* seeds, tree populations were surveyed across the Eastern Cape and KwaZulu-Natal provinces. The wasp was present at 99% of the *S. terebinthifolia* populations with an average of 22% of the seeds being destroyed. In the Eastern Cape Province, the highest seed damage occurred at the start of the winter months, when about 35% of seeds were damaged. This fell to less than 12% in spring and summer when the plants were flowering. *Megastigmus transvaalensis* may have slowed the rate of spread of the plant, but it is unlikely to reduce population sizes of *S. terebinthifolia* in South Africa in the long-term.

Biological control efforts can be assisted by knowing the origin and invasion history of the target species. Genetic analyses are often the only way to elucidate the invasion history of invasive alien plants because it is rare to find detailed records of plant introductions. Both microsatellite and chloroplast DNA analysis were conducted on *S. terebinthifolia* trees from the plant's introduced distribution in South Africa and both Florida and Hawaii, USA. These samples were compared to plants from the native distribution of South America. The analysis indicated that the *S. terebinthifolia* in South Africa was most likely sourced from the state of Rio de Janeiro in Brazil, which is the same source of the invasive populations in Florida and Hawaii. Importantly, the South African populations were all found to be “haplotype A”. Plants samples collected from Hawaii USA were the closest match to the South African plants. Biological control agents known to damage haplotype A which have been considered for use in Hawaii and Florida should therefore be prioritised for South Africa.

Schinus terebinthifolia has a broad distribution in South Africa; however, the majority of the current distribution is limited to the coastal regions along the eastern coast in KwaZulu-Natal Province. This suggests that the species may be climatically limited. Species distribution models in MaxEnt were used to predict the suitable ecological niche of the species. Using occurrence localities from both the native and invaded range to calibrate the models resulted in 56% of the modelled areas being considered suitable for the growth of *S. terebinthifolia* in South Africa. This included areas in the Eastern Cape, Western Cape and Limpopo provinces. When the models were calibrated using just the native range data, or just the invaded range data, predicted distributions were more restricted and limited to the coastal areas of the Eastern Cape and KwaZulu-Natal provinces. The coastal areas between Florianopolis and Santos in Brazil were highlighted as the most climatically similar to the invasive populations of *S. terebinthifolia* in South Africa. These areas should be prioritised if native range surveys for potential biological control agents are conducted in South America.

Although the native seed-feeding wasp is damaging to *S. terebinthifolia* in South Africa, the tree is still not under suitable levels of biological control and is likely to spread and increase in density. New biological control agents are therefore required. Genetic and climatic matching has determined where the most appropriate region to collect new potential biological control agents is. The genetic matching data has also indicated that biological control agents that have been released, or are being considered for release, in Hawaii and Florida, are likely to be suitable for the South African plants because they have been shown to be damaging to ‘haplotype A’. These agents should therefore be the first to be considered for release in South Africa.

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1 Chapter 1: General introduction

1.1 Problem statement

Out of its native range *Schinus terebinthifolia* Raddi (Sapindales: Anacardiaceae) is an aggressive, fast growing invader of both natural and disturbed systems. The tree shades out and displaces native vegetation, often forming dense monocultures that reduce the biological diversity of plants and animals (Ewel et al. 1982; Cuda et al. 2006). The tree has invaded more than 20 countries (Morton 1978) including the United State of America (USA) and South Africa (Randall 2000; Wheeler et al. 2001). In South Africa, *S. terebinthifolia* is invading KwaZulu-Natal, Eastern Cape, Limpopo and Mpumalanga provinces (SAPIA 2018). Uncharacteristically for an invasive plant species, in South Africa the tree has acquired a new association with a native seed predator, *Megastigmus transvaalensis* (Hussey) (Hymenoptera: Torymidae) which is usually associated with the *Rhus* genus (Anacardiaceae). *Megastigmus transvaalensis* has also been accidentally introduced in the USA and into the plants native range in South America (Wheeler et al. 2001; Ferreira-Filho et al. 2015). The wasp is causing important damage to seeds of *S. terebinthifolia* in Florida and Hawaii, USA (Wheeler et al. 2001) and its occurrence in the native range is seen as a threat to the native *S. terebinthifolia* (Ferreira-Filho et al. 2015). The effect of the wasp on the South Africa population of *S. terebinthifolia* is unknown.

There is intraspecific genetic variation within the *S. terebinthifolia* population in the plant's native range (Williams et al. 2005). Additionally, intraspecific hybridisation between two haplotypes in Florida USA occurred, and has resulted in a more aggressive invader than the two haplotypes (progenitors) found in Florida. Impact studies have also indicated that *M. transvaalensis* performs differently between the Florida hybrid and its progenitors (Geiger et al. 2011). Information on the haplotypes found in South Africa are not known and whether the impact of the wasp is affected by a potential hybrid is also undetermined. Identifying haplotypes or hybrids found within South Africa would therefore aid in the management of the species. Additionally, identifying the introductory history of the species will assist South African researchers in developing a biological control programme against it (Gaskin et al. 2011).

In South Africa, the *S. terebinthifolia* distribution is currently limited to regions along the eastern coastline (SAPIA 2018). This suggests that climate may be driving the distribution of this species. Knowing regions suitable for *S. terebinthifolia* will assist in identifying regions where the species shouldn't be introduced as well as areas where management should be prioritised. Additionally, with regard to weed biological control, knowing a species' distribution in the invaded range can assist in prioritising areas that are climatically similar in the native range. This allows researchers to prioritise native range surveys to areas that are climatically representative and thus ensuring collected potential biological control agents are climatically adapted.

This study aimed to investigate and select drivers behind the invasion of *S. terebinthifolia* in South Africa, with the ultimate goal of providing solutions to facilitate in its management. This was done by quantifying the impact of the native seed-feeding wasp, investigating the genetic structure and origin of the South Africa populations of the weed, determining the role of climate in shaping the plants distribution, and prioritising where the most climatically suited potential biological control agents could be collected. In this chapter, a background to biological invasions, biological control of weeds, the target weed and its' natural enemies are provided.

1.2 Biological invasions in South Africa

The introduction of alien invasive species into South Africa coincided with the start of colonialism in the country (Pooley 2009). The first records of intentionally introduced exotic species were in 1652, when a Dutch horticulturalist Hendrick Boom started a garden in Cape Town, Western Cape Province for the Dutch East India Company (Pooley 2009). Global trade and travel have since continued to contribute to both intentional and accidental introductions of exotic species (Sala et al. 2000; Keane & Crawley 2002; Richardson et al. 2003; McKinney, 2006). Originally, the intentional introductions of exotic plants were for ornamental use, food, fibre, soil stabilisation and timber purposes (Pimentel et al. 2005; van Wilgen & Richardson 2014). However recent literature suggests unintentional introductions and the horticultural industry are the main contributors to the introduction and spread of alien invasive species (Richardson et al. 2003; Martin & Coetzee 2011; van Wilgen et al. 2012; Faulkner et al. 2017). In South Africa, there have been an estimated 9000 introductions of alien plants, of which about 200 are listed as invasive (Macdonald et al. 2003; Richardson et al. 2011). The success of invasive species establishing and spreading in South Africa means it is one of the most invaded countries in the world, with both natural and semi-natural ecosystems seriously affected (Richardson et al. 1997; Moran et al. 2013). South Africa has more invasive alien trees per square kilometre than any other country (Henderson 2001). These invasive plants negatively affect biodiversity conservation (Mooney 2005; Vellend et al. 2007) and therefore need to be managed and controlled (van Wilgen et al. 2001; Richardson & Kluge 2008).

A number of management strategies exist for invasive alien plant species and South Africa actively manages many invasive species, including woody terrestrial invaders such as *S. terebinthifolia* (van Wilgen et al. 2011). Due to their different growth forms, biology's and the habitats they are invading, management strategies need to be site and species specific. Three different methods of control exist for managing invasive alien plants, namely; mechanical, herbicidal and biological control (DiTomaso 2000; van Wyk & van Wilgen 2002). Integrated control is another method, which encompasses a variety (at least two) of the methods mentioned above. It should be noted, unlike South Africa, in some countries biological control is not accepted as a control option for invasive plants (van Wilgen & Richardson 2014).

1.3 Mechanical control

Mechanical control is the physical removal or destruction of an invasive plant species. The magnitude of the control effort can vary greatly from individual seedlings being hand pulled to large tracks of land cleared with heavy machinery. Mechanical control can be conducted in combination with fire (van Wilgen et al. 2001). In South Africa, fire has been used to manage large stands of Australian *Acacia* species (Richardson & Kluge 2008). Fires often result in the large-scale germination of seeds stored in the seedbank which can then be cleared with follow up clearing methods such as hand pulling (Holmes et al. 2008). Mechanical control methods are usually expensive, have non-target effects and use up valuable person-hours, sometimes with very little success. For example, in South Africa between 1996 and 2008, the South African government spent R435 million on mechanical control of Mesquite (*Prosopis* spp.). Despite this substantial investment, only about 0.6% of the infestation was controlled each year and the weed was able to re-grow into the cleared areas (van Wilgen et al. 2012).

1.4 Chemical control

Chemical or herbicide control involves the use of registered herbicides to kill plants (Edgin 2007). The herbicides can be specific to a group of plants, for example, grasses or broad-leaved species, leaving other plants unharmed. This method is often used in combination with mechanical control. In this scenario, herbicide is applied to stumps or to kill seedlings after felling or burning, which ultimately prevents re-sprouting (Van Wilgen et al. 2001). The size of application, as in mechanical control, can also vary greatly from targeted individual seedlings and cut stumps to large foliar applications sprayed from aircrafts. Unfortunately, the use of herbicides can have negative impacts on the environment because of their toxicity to non-target plants and their residual times in the environment. Plants can also build up resistance to herbicides making them less effective (Powles 2018).

Both mechanical and chemical control methods are expensive, labour intensive and unsustainable for large-scale infestations of invasive alien plants. This is compounded if mechanical and chemical control measures are poorly executed or not sustained (Holmes et al. 2008).

1.5 Biological control

When a plant is introduced outside of its' native distribution the natural enemies which usually feed on it, such as herbivorous insects and fungal pathogens, are usually left behind. The Enemy Release Hypothesis predicts that this escape from natural enemies results in the introduced plant having a competitive advantage over native plants in the introduced range (Williamson 1996; Crawley 1997). The Enemy Release Hypothesis is based on three fundamental assumptions: 1) natural enemies regulate the abundance of native plant species; 2) natural enemies have a greater impact on native species than on invasive species, and 3) invasive plants benefit from the reduction of regulator natural enemies (Keane & Crawley 2002). Biological control aims to reverse this enemy release. It involves the intentional importation of natural enemies (biological control agents), native to the same geographical region as the target weed species. The intention is to establish the natural enemies in the invaded distribution, and restore the natural balance existing between the two organisms to below a certain threshold (De Bach 1964; Harley & Forno 1992; Muller-Scharer & Schaffner 2008; Van Driesche et al. 2010). Biological control is regarded as a cost effective and environmentally friendly method of controlling invasive weeds. The use of biological control can reduce the use of herbicides and mechanical control and provide long-term and sustainable control in both agricultural and natural ecosystems (McFadyen 1998; Fowler et al. 2000; Clewley et al. 2012).

One of the primary concerns with regard to weed biological control is the risk of non-target impacts by biological control agents (Simberloff & Stiling 1996; McFadyen 1998). Once an agent is released it becomes a permanent part of the ecosystem and the changes that the agent makes to the ecosystem must be positive. For this reason, biological control agents must be host specific, so that they will only feed on the target weed and not on indigenous or economically important plant species. It is also important that biological control agents are suitably damaging to the target weed. The release of ineffective biological control agents should be avoided whenever possible because there is some innate risk in every new release (McClay & Balciunas 2005). Biological control agents must therefore be shown to be suitably host specific and damaging prior to release.

A measure of the safety and efficacy of weed biological control can be made by interrogating past biological control programmes. Detailed analyses of past weed biological control programmes have clearly indicated that if done correctly, following standard procedures, the risks of non-target effects from biological control agents are minimal (Funasaki et al. 1988; Fowler et al. 2000; Pemberton 2000; Paynter et al. 2004; Suckling & Sforza 2014; Moran & Hoffmann 2015). It is estimated that less than 1% of all the agents released globally have had a negative impact on non-target plant populations (Suckling & Sforza 2014) and in all these cases the non-target impacts were predicted by host specificity testing (Downey & Paterson 2016). The biological control programmes that resulted in non-target feeding are predominantly older projects and the agents would almost certainly not be released under current regulations (Groenteman et al. 2011; Hinz et al. 2014; Suckling & Sforza 2014). Within the countries practicing biological control, 468 biological control agents have been intentionally released worldwide for the control of 175 weed species in 48 plant families (Schwarzländer et al. 2018). Of the 313 species for which impact could be categorized, 172 (55.0%) caused medium, variable or heavy levels of damage (Schwarzländer et al. 2018). Across all countries and regions, 65.7% of the weeds targeted for biological control experienced some level of control (Schwarzländer et al. 2018). Even with the impressive success rate and the very limited risk associated with weed biological control, it is not implemented globally (Shaw et al. 2018).

The main countries practicing biological control are Australia, New Zealand, Canada, USA and South Africa. South Africa is one of the most active countries with regard to research and implementation of weed biological control (Moran & Hoffmann 2015). Biological control was first implemented in South Africa with the introduction of a cochineal insect against a prickly pear cactus *Opuntia monacantha* Haw. (Cactaceae) in 1913 (Moran et al. 2013). Since this first release, 93 species of insects, mites and plant pathogens have been established on 59 IAP species in the country (Zachariades et al. 2017). An additional 25 species of plants have been worked on, or are currently being investigated, but do not have biological control agents established on them yet (Zachariades et al. 2017). In an economic assessment comparing the costs of biological control research and implementation in South Africa to the benefits of restored ecosystem services, de Lange & van Wilgen (2010) showed biological control to be extremely beneficial in economic terms with estimated benefit to cost ratios from 8:1 up to 3726:1. The main benefit of biological control programmes is that they are self-sustaining, so the benefits continue to be accrued every year after the release of the agent.

1.5.1 Stages of a biological control programme

Schinus terebinthifolia is a new potential target for biological control in South Africa. The steps in a typical biological control programme involve pre-release assessments in the introduced distribution; surveys for potential agents in the native distribution; importation of potential agents into quarantine facilities and host specificity testing; followed by the release of the agent and post-release evaluation (McFadyen 1998; Van Klinken & Raghu 2006). This study covers part of the pre-release components of the biological control pipeline for *S. terebinthifolia,* including pre-release surveys in the introduced distribution and prioritising where agents should be sourced through genetic and climatic matching.

Before starting a biological control program, it is important to determine whether potential agents are not already present on the target weed within the introduced distribution and this can be achieved by conducting a pre-release survey (Dudley et al. 2008). Some insects or other natural enemies may have moved with their target species or can be introduced unintentionally at a later stage. In these scenarios collecting and testing the same species from the native range would be a waste of both resources and time (Dudley et al. 2008; Canavan et al. 2018). For example, *Robinia pseudoacacia L.* (Fabaceaea) was introduced into Europe from the Appalachian mountain range in USA 400 years ago (Cierjack et al. 2013). Since its introduction into Europe, four phytophagous insects associated with *R. pseudoacacia* have made it across to Europe where they effect *R. pseudoacacia* populations (Cierjack et al. 2013). Similarly, in South Africa, the biological control agent *Teramesa romana* Walker (Eurytomidae:

Chalcidoidea) was found to be present in South Africa prior to the initiation of the biological control programme (Canavan et al. 2018). With regard to *S. terebinthifolia* in USA, prior to overseas exploration for potential agent, a pre-release survey was conducted. This survey resulted in the discovery of a wasp species later described as *M. transvaalensis,* which feeds on the seeds of *S. terebinthifolia* (Grissell & Hobbs 2000). The primary host plant of *M. transvaalensis* is not from the native distribution of *S. terebinthifolia* but rather from a closely related plant genus (*Rhus*) from South Africa (Grissell & Hobbs 2000). Subsequent studies have shown the wasp to be an important seed-predator of *S. terebinthifolia* in the USA, damaging a significant percentage of the seeds. The same wasp has since been located on *S. terebinthifolia* in South Africa but its impact has not been determined. These pre-release surveys highlight the importance of knowing what is already present in the introduced range prior to exploratory work in the native distribution.

Once the decision to source new biological control agents has been made, the next step is to determine where the most appropriate biological control agents could be sourced. Genetic matching is used to determine the source, or origin, of the invasive alien plant population, so that agents that are adapted to the weed population can be found (Gaskin et al. 2011). There is evidence that genetic matching of the South African weed population of *S. terebinthifolia* will be important for the success of the programme (Williams et al. 2005; 2007; Cuda et al 2019). Climatic matching is also important at this stage, as agents must be sourced from areas that are climatically suitable if they are to survive in the introduced distribution after release (Mukherjee et al. 2012).

Once researchers know where to source potential agents, surveys are conducted in the native distribution and promising potential agents are imported into quarantine for further testing. Agents are prioritised based on the likelihood of them being suitably host specific and damaging (Paterson et al. 2014). It is at this stage of the biological control programme that agents may be sourced from other countries that have already developed biological control agents for the same target weed. Utilising biological control agents that are already being used in other countries can result in a significant reduction in costs and increase the chances of success of the biological control programme (Paterson et al. 2019).

Once a potential agent is imported into quarantine, it must undergo host specificity testing (McFadyen 1998). The process and methods of host specificity testing are continuously refined and improved, but the basic procedure is now considered standard practise in weed biological control (Wapshere et al. 1989; Van Klinken & Edwards 2002; Briese 2006). Host specificity testing aims to determine the host range of a potential agent; this is done by testing its performance on close relatives of the target weed. More and more distantly related plant species are then tested until the host range has been circumscribed (Wapshere et al. 1989; Van Klinken & Edwards 2002; Briese 2006). Essentially host specificity testing provides the basic information upon which the safety of a proposed biological control agent can be assessed (Heard 2002; McFadyen et al. 1998). The results of host specificity testing are the most important component of convincing governments or regulating bodies to allow the release of biological control agents.

Post-release evaluations are an essential, although often neglected, component of any biological control programme (Morin et al. 2009). Post-release evaluations quantify the success of biological control, allowing researchers to determine whether further biological control agents are required to control a weed and whether the resources that were used to develop the biological control agent were justified. One of the most important contributions of post release evaluations is that they can provide an economic evaluation of the benefit of biological control (Morin et al. 2009; Hayes et al. 2013; Hinz et al. 2014; Martin et al. 2018).

1.6 Control of invasive trees in South Africa

The control of invasive trees is particularly difficult compared to other invasive alien plants because the costs and difficulty of control increases with the amount of biomass that must be removed from the system (Marais et al. 2004). Even though invasive trees are abundant worldwide, their management is still limited, with relatively few examples of success (van Wilgen & Richardson 2014). South Africa has more invasive trees per square kilometer than any other country (Henderson 2001). It has 17 main invasive tree genera dominated by Australian *Acacia* and *Eucalyptus* species and the European *Pinus* species, but also including *Schinus* sp. (Nel et al. 2004). Chemical and mechanical control has been implemented against all these species, in some cases involving substantial investments, however, in all cases success has been limited (van Wilgen et al. 2012; van Wilgen & Richardson 2014). There are, however, 16 species of invasive tree in South Africa that have been subjected to biological control, and in six cases the level of control achieved has been substantial (Klein 2011). Integrated control using biological control, chemical and mechanical control have also proved successful in gaining large-scale and sustained control of invasive trees in South Africa (Zacharaides et al. 2016). Even though biological control has been shown as the only method of gaining large-scale control of invasive trees in South Africa its implementation can sometimes be limited where conflict of interests exist. The biological control of a number of species is restricted, primarily if they have some form of commercial value. In these scenarios the use of agents that damage the reproductive organs of the trees are prioritised as they can lead to a reduction of seed, while still allowing the species to be exploited commercially for its timber, thus avoiding conflicts of interest (Impson et al. 2011). In some cases, biological control is completely abandoned and not considered, as is the case of *Pinus* species in South Africa (Lennox et al. 2009). Fortunately, no conflict of interest is expected with regard to the management of *S. terebinthifolia* in South Africa as it is only utilised as an ornamental plant and is not grown commercially.

1.7 Study species

1.7.1 *Schinus terebinthifolia* Raddi (Sapindales: Anacardiaceae)

The accepted name of the focal organism of this study is *Schinus terebinthifolia* (The Plant List 2018; GBIF 2019) and this is the name used throughout this thesis; however, *Schinus terebinthifolius* a synonym of *Schinus terebinthifolia* is regularly encountered within the literature. The common name of *S. terebinthifolia* is regionally dependent; some of these names include Brazilian peppertree, Bahamian holly, Florida holly, broadleaf pepper, Brasiliaanse peperboom (Afrikaans) and Christmas berry tree (Orwa et al. 2009)

Schinus terebinthifolia belongs to the *Schinus* genus of the family Anacardiaceae, which has about 80 genera and about 600 species which are primarily trees and shrubs. The cashew, pistachio, mango, sumac and poison oak are commercial species within the family (Hight et al. 2002). The genus *Schinus* has 28 species and its centre of distribution is northern Argentina (Barkley 1944; 1957). In South America, Barkley (1944) recognised five varieties of *S. terebinthifolia* namely, *S. terebinthifolia* var. *terebinthifolia* Raddi from Venezuela to Argentina; *S. terebinthifolia* var. *acutifolius* Engl. from southern Brazil and Paraguay to Missiones in Argentina; *S. terebinthifolia* var. *pohlianus* Engl. (the most common variety of the species) from southern Brazil to Paraguay and northern Argentina; *S. terebinthifolia* var.

raddianus Engl. from south-central Brazil; and *S. terebinthifolia* var. *rhoifolius* (Mart.) Engl. from south-central Brazil (Hight et al. 2002). Visually, varieties are difficult to differentiate based on their morphologies (Ferriter 1997). Additionally, Williams et al. (2005) investigated the native genotypes of *S. terebinthifolia* and found 10 different haplotypes (A-J) distributed within the plants native range as well as a hybrid between two Haplotypes found invading Florida, USA.

Schinus terebinthifolia is a evergreen tree growing up to six meters in height. The tree has a strong trunk with spreading, horizontal branches that form a dense round or oval-shaped crown (Henderson 2001; Lenzi & Orth 2004a). The tree's leaves are dark green and have prominent pale veins. The flowers are small and white and appear from September to March in South Africa. The tree is primarily a dioecious species, with male and female flowers occurring on separate trees, although some monecy is known (Ferriter 1997). *Schinus terebinthifolia* is a prolific seed producer, with bright red drupes surrounding a single internal seed (Dlamini et al. 2018). In South Africa drupes are primarily found during the winter but in the USA fruits have been shown to persist on trees for up to eight months (Ferriter 1997).

As early as 1919, planting of S. *terebinthifolia* was recommended for ornamental, hedging, and shade purposes in South Africa (Potts 1919; Sim 1919). However, the first official record of naturalised *S. terebinthifolia* in South Africa is from the 1980's from sites in KwaZulu-Natal Province (SAPIA 2008; Figures 1.1 and 1.2). The species now has a broad distribution in South Africa, having been recorded as escaping cultivation in four provinces (KwaZulu-Natal, Eastern Cape, Gauteng and Limpopo provinces) (NEMBA 2014). The vast majority of sites (over 90%) are located along the KwaZulu-Natal coast line (Figure1.1).

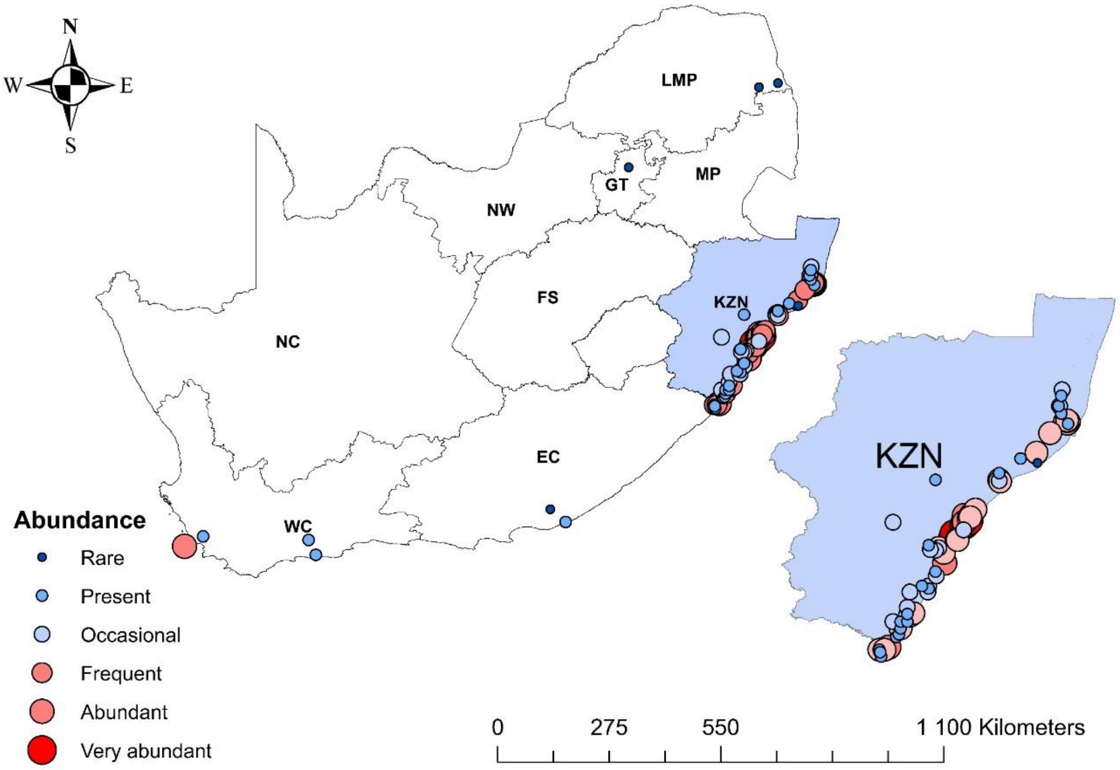


Figure 1.1 Relative abundance of *Schinus terebinthifolia* at recorded sites in South Africa (SAPIA, 2008).

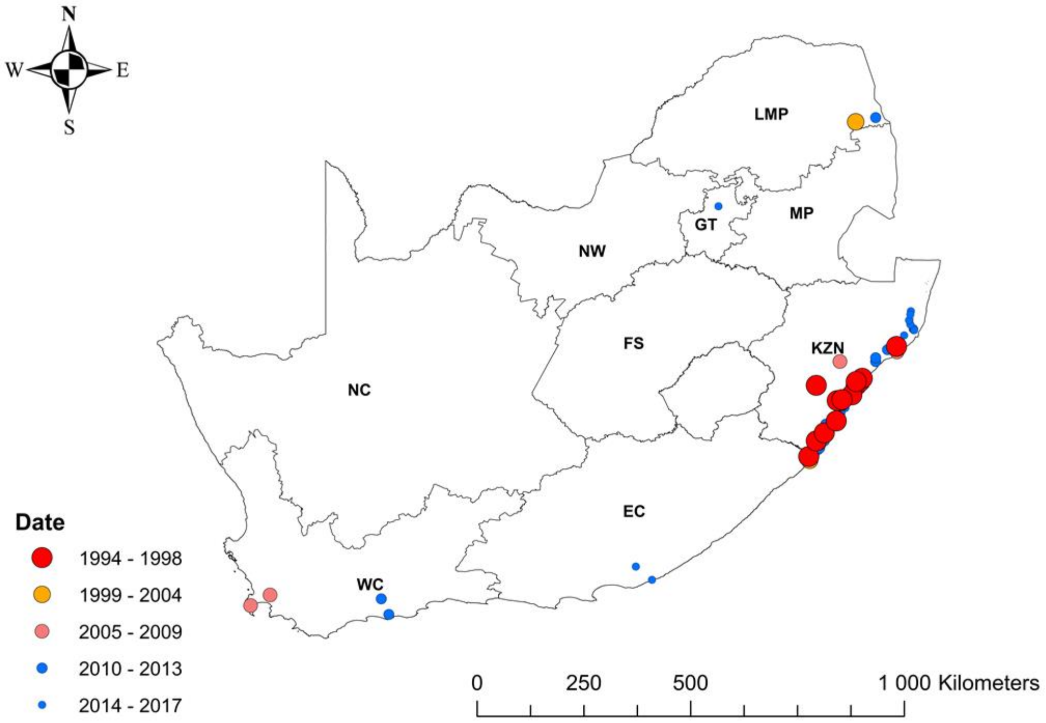


Figure 1.2 First records of *Schinus terebinthifolia* populations in South African populations (SAPIA, 2008).

Schinus terebinthifolia has proved difficult to control in KwaZulu-Natal Province which is the only province where control has been attempted. The tree has a number of characteristics which make it an excellent invader and difficult to control. Namely, it has a high growth rate, prolific seed production, a high germination rate, shade tolerant seedlings, and the bright drupes readily attract seed dispersing birds and mammals (Stone et al. 1992).

In some countries, this tree has beneficial attributes and commercial value. In the native range the tree is recommended for land reclamation (Ferreira-Filho 2015), due to its pioneer properties and attractiveness to birds (High et al. 2002) and its ability to develop in soil with low nutrient levels (Ferreira-Filho 2015). Also in the native range, the leaves, bark, seeds, resin and oleoresin of *S. terebinthifolia* are used as medicine (Gundidza et al. 2009). Out of the plants native range, records exist for where the tree has been used for medicinal, cosmetic, and culinary purposes (Barbosa et al. 2007; Guzzo da Silva et al. 2015). It is common in many countries to see the fruits and leaves used in wreaths during Christmas periods (Grissell & Hobbs 2000). In South Africa, concentrated liquid from boiling leaves is inhaled for colds, hypertension, depression and irregular heartbeat (Gundidza et al. 2009). In Zimbabwe a neighbouring state to South Africa, concentrated liquor from crushed leaves or bark is drunk to relieve bronchitis and rheumatism (Wild et al. 1972). The use of *S. terebinthifolia* drupes as red peppercorns for culinary purposes is the most common utilisation of the species and is probably what has led to the broad global distribution of the tree (Habeck et al. 1989; Grissell & Hobbs 2000).

1.7.2 Implemented control measure for *Schinus terebinthifolia* Raddi

In invaded areas of South Africa, the tree has either been left unmanaged or control has been attempted with mechanical (botanical society 2018). Large-scale control has not been achieved and the tree continues to spread as evident from the growing number of records on the SAPPIA database (L. Henderson pers. comm. *Plant Protection Research Institute PPRI-ARC*) (Figure 1.2). These results are similar to Florida where extensive mechanical and chemical control methods used for a number of decades have only provided a temporary solution for the plant (Doren & Jones 1997; Hight et al. 2002; Cuda et al. 2013). In 2011, Florida spent an estimated $1.7 million on control of *S. terebinthifolia,* and over $10.5 million was spent from 1998-2006 with limited success (Rodgers et al. 2012). Biological control has only been attempted in Hawaii however there are pending petitions for the release of potential biological control agents into Florida USA (Hight et al. 2002; Cuda et al. 2019).

1.7.3 Biological control of *Schinus terebinthifolia* in the USA

Work for potential biological control agents for *S. terebinthifolia* was initiated in Hawaii in the 1950s and resulted in the release of three insect species, of which only two established (Krauss 1962; 1963; Hight et al. 2002). In Florida, explorations for biological control agents in South America were initiated in 1987 (Bennett et al, 1990). A large arthropod fauna (139 species) was found associated with the plant in Brazil. Further surveys conducted by researchers of the University of Florida and the Universidade Federal do Paraná in Curitiba, Brazil have increased the insect fauna to at least 200 species (Bennett et al. 1990; Bennett & Habeck 1991). the 1980s and 1990s (McKay et al. 2009). Several insects were identified from exploratory surveys in Brazil during the 1990s as potential biological control agents. Two of the insects: a defoliating sawfly *Heteroperreyia hubrichi* Malaise (Hymenoptera: Pergidae), and a shoot feeder thrips *Pseudophylothrips ichini* Hood (Thysanoptera: Phlaeothripidae) were collected and introduced into Florida quarantine in 1994 to conduct host-specificity testing by J. Medal. (Medal et al. 1999), further surveys were conducted. Wheeler et al. (2016) conducted surveys from 2005-2014 in South America and reported about 150 herbivore species that may be associated with *S. terebinthifolia.* A subsequent survey in Brazil found 120 phytophagous species; over 40 of these species were considered suitable control agents and further investigation of their host ranges were conducted (Wheeler et al. 2016). Explorations conducted in Argentina by McKay (2009) revealed 36 phytophagous insect species and one fungi associated with *S. terebinthifolia.* Species that were considered for release in the USA and that could potentially be effective in South Africa are discussed in Chapter 5.

In the USA an unintentional introduction of seed-feeding wasp, *M. transvaalensis* from South Africa has provided certain levels of biological control (Wheeler et al. 2001). The wasp is native to South Africa where it is known to be a seed predator of *Rhus* species. In Florida the overall seed mortality caused by this wasp has been reported to be as high as 76% (Wheeler et al. 2001). Similar results were recorded in Hawaii with 80% seed mortality (Hight et al. 2002). The impact of the wasp on *S. terebinthifolia* in South Africa has not yet been considered. However, a study done in the Western Cape Province by Iponga (2009) recorded the same wasp feeding on the closely related *S. molle*, reducing the pre- and post-dispersal survival of *S. molle* seeds.

South Africa is currently considering a biological control programme against *S. terebinthifolia*. The extensive and continuing biological control programme in Florida will significantly contribute to developing a biological control programme. Previous biology, impact and host specificity work done in USA can be used to prioritise agent appropriate for South Africa.

1.8 Objectives of the study

The aim of this study was to determine whether biological control of *S. terebinthifolia* is required and, if it is required, where the most effective biological control agents could be sought. The first objective, reported on in Chapter 2, was to determine the distribution and impact of the seed-feeding wasp *M. transvaalensis.* Knowing if the wasp is contributing to management of the tree in South Africa will assist when selecting potential biological control agents. In Chapter 3, the origin and population genetics of *S. terebinthifolia* were investigated. *Schinus terebinthifolia* has a number of haplotypes and a known hybrid, and knowing which haplotype and/or hybrid are invading South Africa is important for all management strategies, and for biological control in particular. In Chapter 4, climatic matching was conducted to determine where the most climatically suitable biological control agents could be sourced for the plants native distribution. Finally, in Chapter 5, the importance of genetic and climate matching in biological control programmes are discussed, potential biological control options for *S. terebinthifolia* in South Africa are considered, and management priorities are suggested.

2 Chapter 2: Quantifying predation of *Schinus terebinthifolia* seeds by *Megastigmus transvaalensis*

2.1 Introduction

One of the reasons for the success of invasive species out of their native range is an escape from natural enemies. Any species leaving its indigenous range escapes a number of controlling mechanisms including plant herbivores, diseases and other natural enemies. If environmental conditions are suitable, this can result in a rapid increase in distribution and abundance and the plants becoming invasive (Keane & Crawley 2002). This hypothesis forms the conceptual basis for classical weed biological control programmes, where deliberate introductions of the invasive species' herbivores have been shown to have controlling effect on populations of invasive plants. Biological control agents are usually chosen from the insects or diseases that feed on the plant in their shared native distributions.

However, in some cases, herbivores that feed on close relatives of that target weed can move onto the target weed in the introduced distribution and may provide some level of control. These herbivores have no evolutionary history with the target weed, making them very different to typical biological control agents. Such 'new association' can even lead to a greater impact on the weed than that provided by herbivores with long evolutionary associations (Robinson & Wellborn 1988; Hokkanen & Pimentel 1989; Baltz & Moyle 1993; Creed & Sheldon 1995; Trowbridge 1995). New associations between invasive plants and potential biological control agents are typically generated as part of experiments carried out during biological control programmes, but on rare occasions invasive plant species are colonised by species native to the new range of the invasive species. An example is seen in South Africa where the South American *S. terebinthifolia* and *S. molle* have been accepted by a native seed predator *M. transvaalensis* (Grissell & Hobbs 2000; Iponga et al. 2008).

The impact and distribution of the wasp on *S. terebinthifolia* are unknown in South Africa. It is assumed that because South Africa is the indigenous range of the species the primary hosts may provide refuges for when *S. terebinthifolia* seeds are not available for the wasp, thereby allowing numbers to increase as seen in *S. molle* (Iponga et al. 2008). The aims of this study are to determine the impact of *M. transvaalensis* on *S. terebinthifolia* and to see if there are geographical or temporal differences in its abundance. This information may prove vital when prioritising biological control agents because if *M. transvaalensis* damages enough fruits there would be no need to consider alternative fruit feeding agents such as *Lithraeus atronotatus* Pic (Coleoptera: Bruchinae) a fruit feeding bruchid known from the tree's native range (McKay et al. 2009).

2.2 Study Species

2.2.1 *Megastigmus* Dalman (Hymenoptera: Torymidae)

Subfamily *Megastigminae* of the Torymidae has over 160 described species, with one-third phytophagous species specialising in feeding on seeds, one-third parasitoides of gall-forming insects, and one-third having unknown host records (Grissell 1999; Noyes & Sadka 2003). Forty-nine species of the seed feeders are associated with conifers and 21 species develop in seeds of five families of Angiosperms (Rosaceae: 13 spp.; Anacardiaceae: four spp.; Fabaceae: two spp.; Aquifoliaceae: one spp.; Hamamelidaceae: one spp (Roques et al. 2016). *Megastigminae hypogeus* (Hussey), *M. thomseni* (Hussey), and *M. transvaalensis* (Hussey) all attack the seeds of Anacardiaceae (Hussey 1956; Grissell & Prinsloo 2001).

2.2.2 *Megastigmus transvaalensis* (Hussey) (Hymenoptera: Torymidae)

Megastigmus transvaalensis was synonymised in 2001 from two species, *Eumegastigmus transvaalensis* (Hussey) and *Eumegastigmus rhusi* (Hussey) (Grissell & Prinsloo 2001; van Noort 2018). *Megastigmus transvaalensis* is a seed-feeding chalcid wasp native to South Africa that was first described in 1956 by Hussey on seeds of the exotic red pepper tree *S. molle,* a sister species of *S. terebinthifolia,* and was first recorded on *S. terebinthifolia* in 1998 (Grissell & Hobbs 2000; Scheffer & Grissell 2003). The wasp has also been recorded utilising another *Schinus* species, *Schinus polygamus* (Sapindales: Anacardiaceae) in Chile (Pujade-Villar & Caicedo 2010; Fernandes et al. 2014).

Megastigmus transvaalensis is found in most parts of the world where *Schinus* is present (Roques et al. 2010; Liebhold et al. 2012). To date the wasp is found in Florida, California and Hawaii, USA (Wheeler et al. 2001; Scheffer & Grissell 2003), the Canary Islands (Grissell 1979), Reunion, Mauritius (Habeck et al. 1989), Brazil (Ferreira-Filho et al. 2015) and Argentina (Wheeler et al. 2001). Possible reasons for this widespread distribution is the poorly regulated trade of *S. terebinthifolia* seeds as red peppercorns of *Schinus* species (Auger-Rozenberg & Roques 2012). Regulations concerning the trade in tree seeds are not in place in a number of countries. Trade through the internet is developing, allowing seeds to move quite freely all over the world (Roques et al. 2016).

2.2.3 Basic Biology

The complete life history of *M. transvaalensis* has not been investigated however, some biological information is known and some is assumed from similar species (Cuda et al. 2008). Adult *M. transvaalensis* are pale yellow-brown in colour (Figure 2. 1 a & b). Males range in size from 2.3 to 2.9 mm whereas females tend to be larger. Body length for female wasp ranges from 3.1 to 3.4 mm. The length of the abdomen and ovipositor range from 1.2 to 1.4, and 1.5 to 1.9 mm respectively (Cuda et al. 2008). Almost half of the overall body length in females is due to the ovipositor (Figure 2.1a) (Van Noort 2018).

Figure 2.1 Female (left) and male (right) *Megastigmus transvaalensis* (Hussey) (Hymenoptera: Torymidae) (photos: van Noort 2018).

Gravid females of *M. transvaalensis* may lay up to 10 to 25 eggs (Cuda et al. 2008). The eggs are usually deposited individually within a *S. terebinthifolia* drupe however more than one may be deposited, larvae are cannibalistic and therefore usually only one larvae will complete its development within a drupe. There are five instars, the same number of instars reported for *M. nigrovariegatus* Ashmead (Cuda et al. 2008). Larvae transform into pupae within the drupe and may remain in the drupe for many months in a stage of prolonged diapause. Prior to emergence of the adult wasp, it is difficult to distinguish between infested and uninfested drupes, as there is no external evidence of the insect developing inside the seed. Adult emergence is determined by photoperiod, which usually coincides with the flowering phase of *S. terebinthifolia* (Wheeler et al. 2001).

2.3 Methods and Materials

2.3.1 Distribution and Impact

A field trip was conducted to sites within the distribution of *S. terebinthifolia* in South Africa during May 2016 (Figure 2.2). Over 60 populations within the Eastern, Western Cape and KwaZulu-Natal provinces were visited. At each site, drupes were collected from a single randomly selected tree within the population. The drupes were then placed in a paper bag, labelled with tree number, geographical co-ordinates and region of occurrence. The drupes were later examined in the laboratory for seed predation by counting the number of intact and externally damaged seeds under a dissecting microscope. The drupes were then placed in emergence jars under a 12:12 photoperiod artificial light regime at 20°C to stimulate wasp emergence. After four weeks, the drupes were re-checked for emergence and the remaining seeds were dissected to look for larvae or pupae.

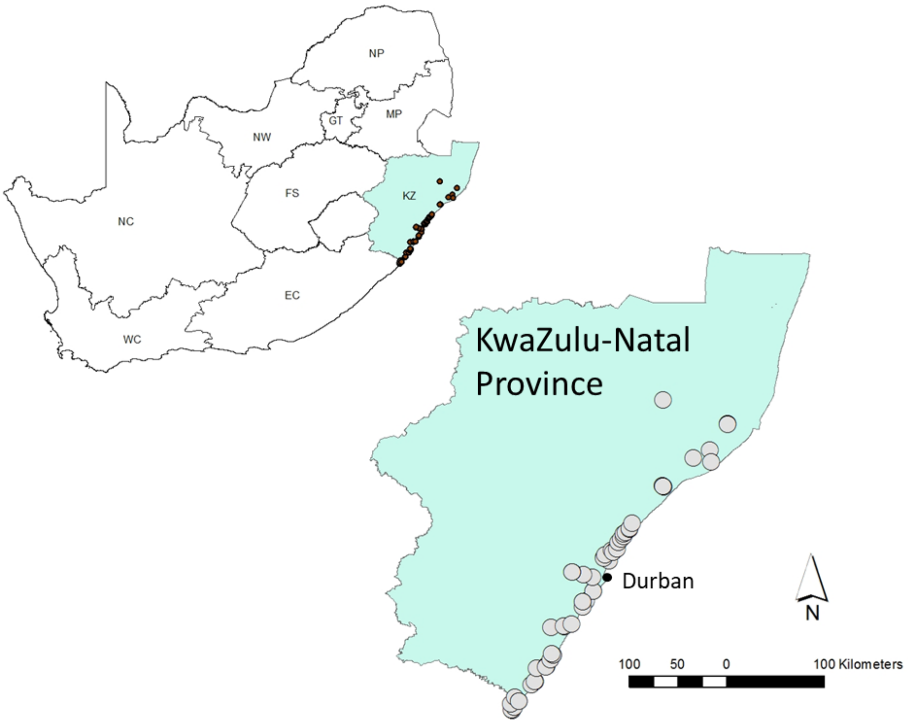


Figure 2.2 Localities of sampled *Schinus terebinthifolia* in KwaZulu-Natal Province, South Africa

2.3.2 Monthly sampling

In order to quantify seasonal change in *M. transvaalensis* impact on *S. terebinthifolia*, as well as if there were site-specific differences, repeated surveys were conducted at four sites in the Eastern Cape Province (Figure 2.3). Surveys were conducted during May, August, October and December in 2016 and January, February, March, April, May, June, July, and August in 2017. The number of fruit on the trees was visually estimated and the presence or absence of flowers noted.

At each site, drupes were collected from randomly selected trees (n = 7). Only mature bright red drupes were collected (Wheeler et al. 2001). The drupes were placed in paper bags, labeled with the tree number, site and co-ordinates. They were later examined in the laboratory under a dissecting microscope. One hundred drupes were randomly selected and checked for predation. Insect-damaged drupes were separated from other damaged drupes using the presence of insects inside the seeds or the presence of emergence holes as indicators. Insect-damaged drupes were kept in a container for four weeks post sampling, and where covered with a cotton screen for aeration until the insects emerged. The insects that were recorded from the seeds were photographed and where necessary samples were sent to Iziko Museum, Cape Town, South Africa for identification. After four weeks, the seeds were re-checked for predation and the damaged drupes counted. The remaining drupes were individually dissected to check for any dead or diapausing larvae and pupae.

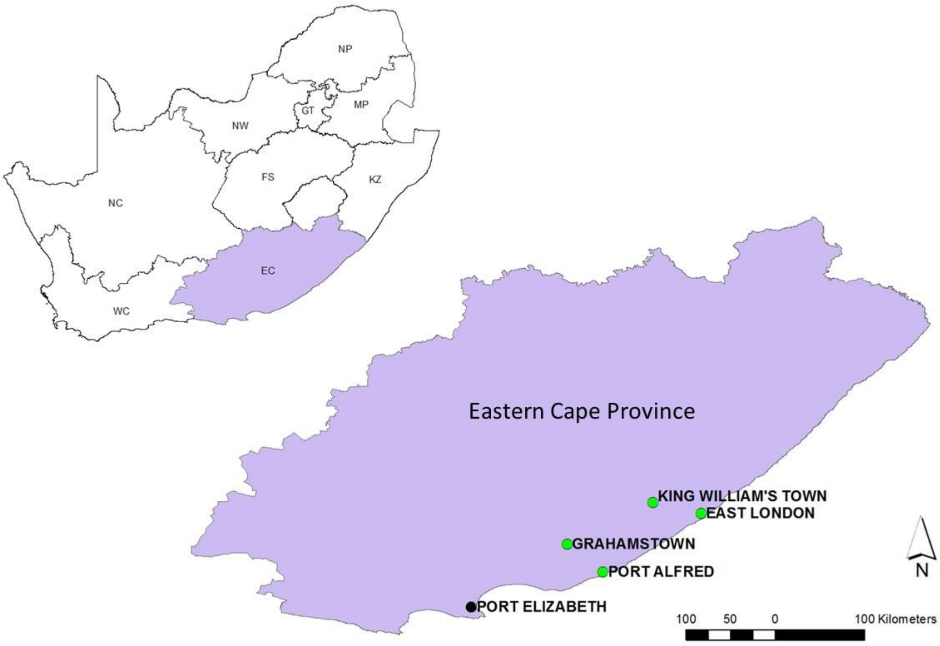


Figure 2.3 The locations of four repeatedly surveyed populations of *Schinus terebinthifolia* in the Eastern Cape Province of South Africa.

2.3.3 Germinations trails

During each sampling event, more than the required 100 seeds for the damage assessment were collected. These extra seeds were used for germination trails using the Tetrazolium Test (Verma & Majee 2013). One-hundred seeds were collected for Eastern Cape sites and 50 seeds from sites in Kwa-Zulu-Natal. Seeds were cut in half and placed in a glass with tetrazolium solution covered by aluminium foil to avoid light and placed in an incubator 20 °C for 12 hours. The liquid was drained and stained half seeds were counted under a microscope. Actively respiring tissues stain pink with this test. Only healthy, undamaged seeds were used in this experiment as it intended to calculate the percentage of viable seeds without the damage of *M. transvaalensis*.

2.3.4. Data analysis

The sampled populations within towns were grouped with each town regarded as a single study site. Descriptive statistics were generated using Microsoft Excel. A factorial ANOVA was conducted to determine differences between regions and between the sampling periods. Post-hoc pair-wise comparisons were conducted to calculate any significant differences. The germination trails were conducted on grouped drupes taken over the whole sampling period, so the variance could not be calculated.

2.4 Results

In total 26300 seeds examined from 74 sites in South Africa. Of these 20.8 % were damaged by *M. transvaalensis.* The only seed attacking herbivore that was encountered during surveys was *M. transvaalensis,* with no other species being recorded and no seeds damaged by other natural enemies.

2.4.1 KwaZulu-Natal Province

Forty-seven sites were sampled across KwaZulu-Natal Province (Figure 2.2). The highest number of damaged drupes by *M. transvaalensis* was 60%, which were recorded at Station Rd Mzumba 62 (-30.621250; 30.54368). There was only one site, Dube Village (-29.7018; 30.9715), where no damage was recorded. On average 23.1% of drupes were damaged and there was no apparent variation in the distribution of the wasp as it was found in 46 of the 47 seven sites sampled across the known invaded range in KwaZulu-Natal Province.

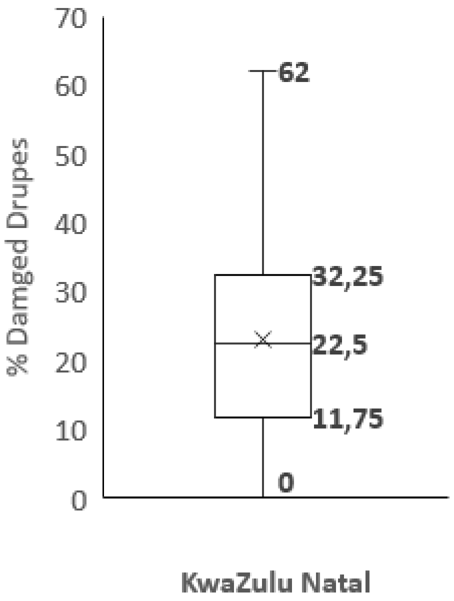


Figure 2.4 Numbers of damaged *Schinus terebinthifolia* drupes across samples sites in KwaZulu-Natal Province, South Africa (n=47 trees). Maximum and minimum damage levels are indicated and the Mean is marked with an X

2.4.2 Eastern Cape Province

Monthly sampling in the Eastern Cape Province recorded the highest damage as approximately 35% in four populations during May of 2016. The lowest number of damaged seeds by *M. transvaalensis* was in December in Grahamstown where 15 % damage was recorded (Figure 2.5). No fruits could be sampled during January 2017 and April 2017 as the trees were flowering and no suitable fruits were available. There was a general decrease in damage from the start of winter leading towards spring and summer. This coincided with the amount of fruit present on the trees.

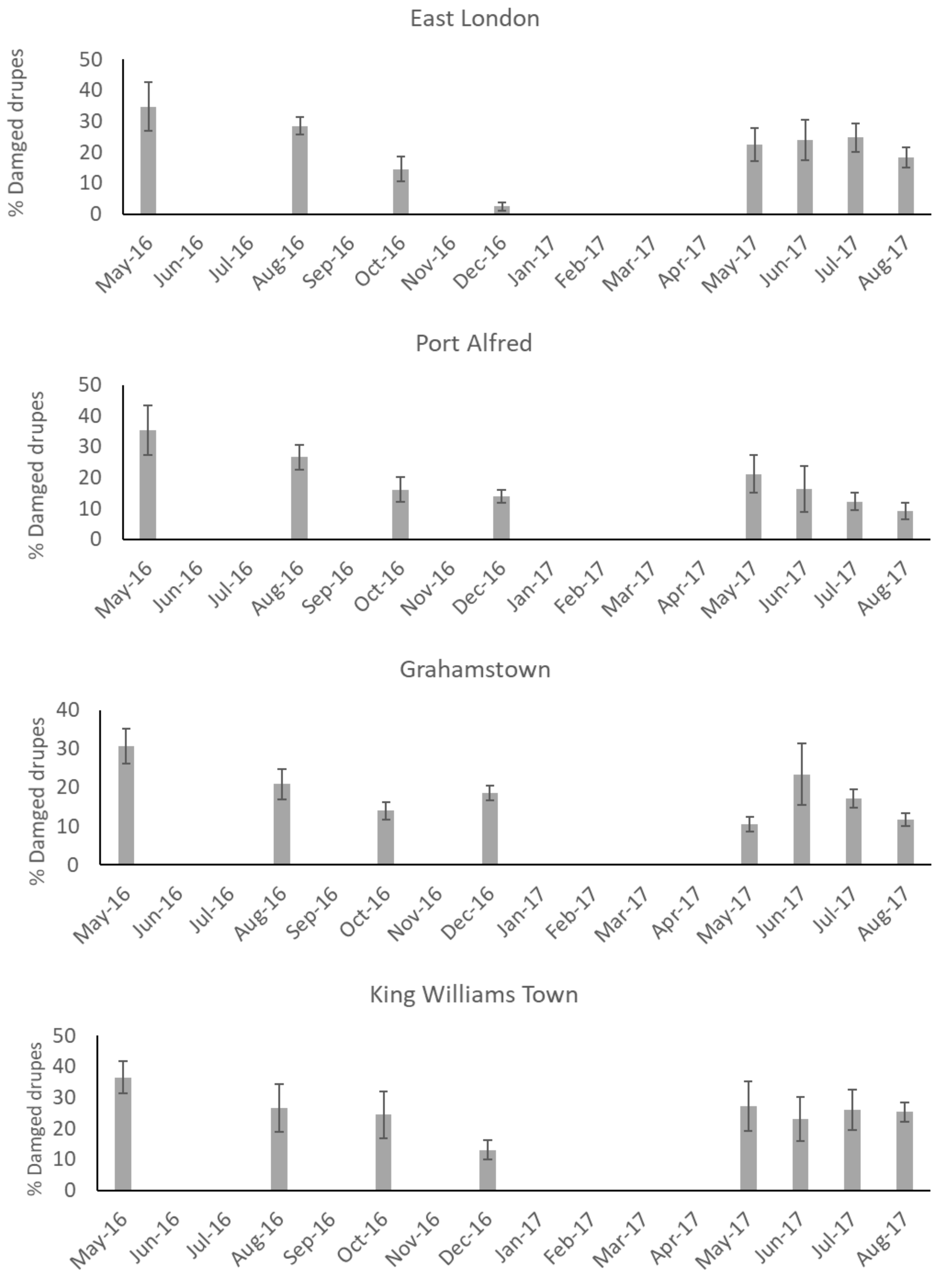


Figure 2.5 The number of *S. terebinthifolia* drupes damaged by *M. transvaalensis* in Eastern Cape Province: East London, Port Alfred, Grahamstown and King Williams Town. At only two sampling events were there significant differences in the damage between the sampling localities. These were in December 2016 where there were significantly less drupes damaged in East London

than the other three sampling localities (F=10.9; *P*=0.001) (Figure 2.5). There were also significantly less drupes damaged in August 2017 at East London, Port Alfred and Grahamstown compared to King Williams Town (F=6.4; *P*= 0.0002). There was no difference between the four sites during the rest of the sampling periods (P >0.05) (Figure. 2.6).

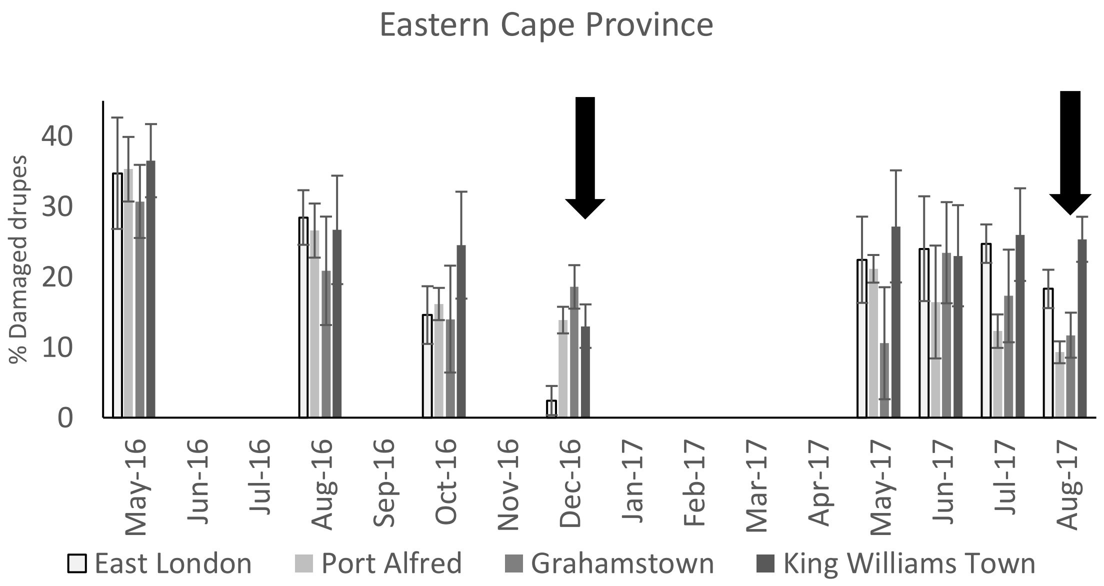


Figure 2.6 The damaged *Schinus terebinthifolia* drupes on different dates at each of the four study populations in the Eastern Cape Province (East London, Port Alfred, Grahamstown and King William's Town). Arrows indicate significant differences between sites. Error bars represent standard error.

Data were combined to give an indication of the trends in the Eastern Cape Province over time (Figure 2.7). No fruit were present from January to April when the plants were flowering. There were significantly less damaged seeds in October and December, May and August compared to the other months sampled where there were drupes available. There were no statistical differences between the rests of the months (F= 13.7; P > 0.05) (Figure 2.7)

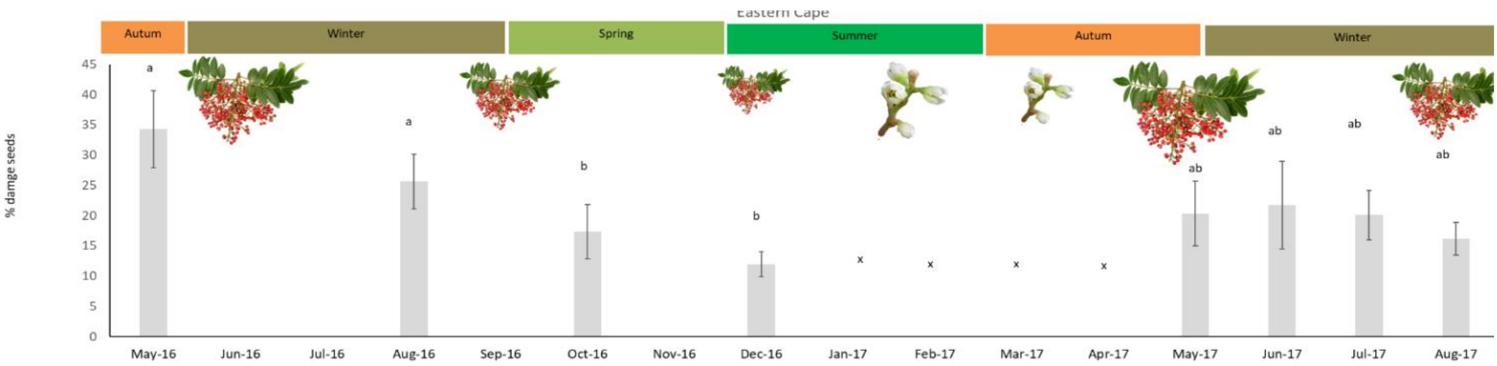


Figure 2.7 The percentage damaged *Schinus terebinthifolia* drupes from sites surveyed across the Eastern Cape Province. Pictures indicating amount of available fruits and flowers as well as the season. Error bars represent standard error letters indicate statistical difference.

The highest germination rate of drupes was recorded in from Port Alfred (48%), followed by East London (24 %) and Grahamstown (20%). The lowest germination was recorded in King William's town (13%) (Figure 2.8).

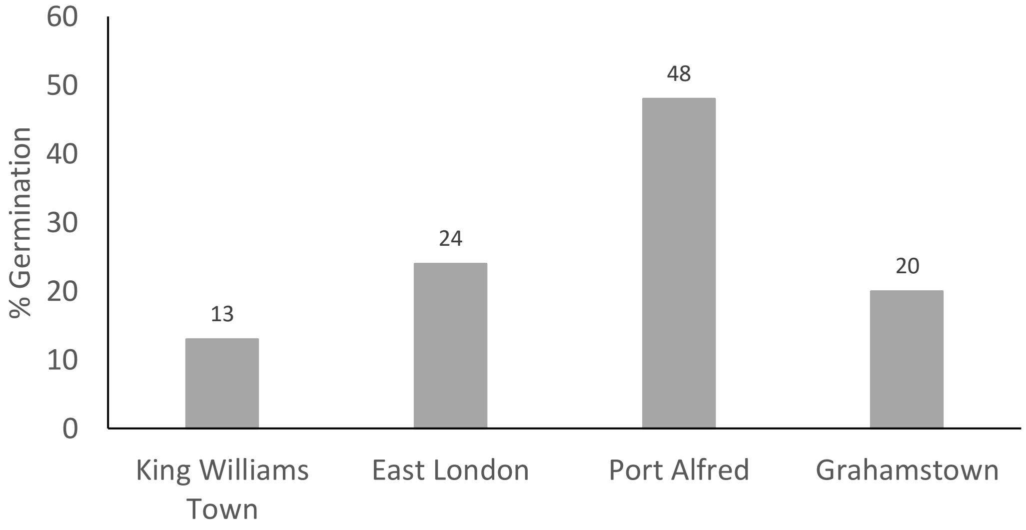


Figure 2.8 Germination of *Schinus terebinthifolia* from sites in the Eastern Cape in East London, Port Alfred, Grahamstown and King William's town in December.

2.5 Discussion

The study revealed that the native wasp *M. transvaalensis* was the only endophagous seed predator of *S. terebinthifolia* in both the Eastern Cape and KwaZulu-Natal provinces. This confirms previous studies that have shown that alien plant species have fewer natural enemies in the introduced range than where they are indigenous (Maron & Vilà 2001; Keane & Crawley 2002; Mitchell & Power 2003). Enemy release may therefore give *S. terebinthifolia* a competitive advantage over indigenous plants in South Africa.

The wasp was found at all sampled locations, indicating a broad distribution in South Africa. Iponga (2009) also found *M. transvaalensis* over a broad distribution; it was recorded in all sampled sites for *S. molle* during a three-year study between the cities of Cape Town and Kimberly separated by 1000 km. A similar broad distribution by the wasp is also seen in the introduced range in the USA (California, Hawaii, and Florida) and South America (Wheeler et al. 2001; Hight et al. 2003; Ferreira-Filho et al. 2015), suggesting the wasp is an excellent disperser and able to survive under many different climatic conditions. The known indigenous host plants of *M. transvaalensis* *R. crenata* Thunb., *R. lancea* L.f., *R. angustifolia* L., and *R. leavigata* L. (Grissell & Hobbs 2000; Cuda et al. 2002), are also widely distributed in South Africa, overlapping with *S. terebinthifolia* and *S. molle* populations (Henderson 2001; Coates-Palgrave 2002).

In the USA, *M. transvaalensis* has been regarded as a beneficial natural enemy for *S. terebinthifolia,* due to its impact on the seeds (Wheeler et al. 2001). The wasp could be assumed to be playing a similar role in regulating plant populations in South Africa. The presence of *M. transvaalensis* in studies done in the USA showed significant damage to *S. terebinthifolia* seeds with mortality as high as 76% in Florida (Wheeler et al. 2001) and 80% in Hawaii (Hight et al. 2003). In Brazil, *M. transvaalensis* is regarded as a problematic invasive species and was found to damage between 1% and 55% of the seeds of *S. terebinthifolia* (Ferreira-Filho et al. 2015). The same high levels of seed predation were not recoded in South Africa, although moderately high damage (35%) was recorded in winter. The presence of matured seeds during the winter months was probably the reason for increased damage, as the wasps are known to deposit eggs on red fruits (Cuda et al. 2002). The average mortality on the seeds in South Africa was 23.1 % (± 9.75 SE), which is low in comparison to Florida and Hawaii (Wheeler et al. 2001; Hight et al. 2003) as well as when comparing with *S. molle* in South Africa, which had seed mortality as high as 75.33 % ± 7.9 in the summer-rainfall region and 32. 80 % ± 7.9 in the winter- rainfall region (Iponga et al. 2008). In the Eastern Cape Province, there were low numbers of *Rhus* sp. or *S. molle* trees in close proximity to the sampled sites, which may have contributed to the low percentage damage recoded on *S. terebinthifolia*. This is supported by Iponga (2009) who stated that the high percentage of insect seed predation in the summer-rainfall region was probably due to the widespread occurrence of the indigenous *R. lancea* at sites where most of the sampling of *S. molle* populations was conducted.

The viability of seeds from all four towns in the Eastern Cape were tested. Port Alfred had the highest percentage, with 48% of seeds being viable. However, the seeds that were tested for viability were not seeds that have been ingested and regurgitated by birds before being tested. Panetta and McKee (1997) have indicated that the fruit of *S. terebinthifolia* have a near-obligate requirement to be ingested and regurgitated before seeds can germinate. They showed that manually removing the pulp, as was conducted in this experiment, increases the chance of germination in the absence of bird regurgitation. The percentage of germination in Port Alfred were similar to a study conducted by Dlamini et al. (2018) who showed 45-58% of *S. terebinthifolia* seeds in KwaZulu-Natal that were planted after being regurgitated by birds to be viable. The study by Dlamini et al. (2018) confirmed the results of Panetta and McKee (1997) showing that whole fruit of *S. terebinthifolia* germinated at a slower rate than seeds that were ingested and regurgitated by birds. Removal of the seed from the fruit by ingestion and excretion or manual means promotes seed germination, and germination rates do not differ between bird-ingested seeds or mechanically peeled seeds (Panetta & McKee 1997). These data suggest that there is an abundance of viable seeds in South Africa despite the damage from the wasp and that these seeds will be dispersed by birds resulting in the spread of *S. terebinthifolia* in the country. Infield germination, survival of germinated seeds and seedling survival were not tested as part of this study and would play an important part in understanding the tree’s demography in South Africa and on how to manage the species.

The movement of the seed predator onto an invasive species does provide an interesting case study looking at new associations. The success of the new association in regulating the exotic species may be dependent on the recruitment rate of the native herbivore and how resistant the weed species is to the possible attack (Tahvanainen & Niemela 1987). The nature of the interaction also depends on the ability of the native species to colonise the new species, factors such as taxonomic relatedness of the exotic and native plants (Tabashnik 1982) and phytochemical similarities amongst the plant species (Bruce et al. 2005). Ehrlich & Raven (1964) proposed that chemical compounds shared by related plants (due to common descent) are what stimulate feeding and oviposition by specialized insects. This is because closely related plant species have similar biochemical, morphological and physiological traits. With many factors affecting possible interaction between native herbivores and exotic plants, the ability of an insect to colonise a new host is therefore typically related to how host specialised it is (Fraser & Lawton 1994). Specialists may be less able to utilise new hosts over their native hosts (Parker 2012). Therefore new associations of monophagous insects are relatively rare (Hokkanen & Pimentel 1989; Trowbridge 1995; Robinson & Wellborn 1988; Baltz & Moyle 1993; Creed & Sheldon 1995). Seeing that *M. transvaalensis* is able to complete its life cycle on a number of *Rhus* species as well as S. *terebinthifolia* or *S. molle* this is a new association of an oligophagous insect. The impact on the tree is not particularly high in South Africa and the tree does not seem to be significantly impacted by the species to the point of reducing its populations. This could be explained by the presence of alternative, and possibly preferred host plants in the native distribution of South Africa which are not present in USA and Brazil.

Regardless of the high rate of damage by *M. transvaalensis* in Florida and Hawaii, biological control has still been pursued because the wasp was not able to bring the tree to an acceptable level of control (Hight et al. 2002; Cuda et al. 2016; Wheeler et al. 2016). The low rate of seed predation in South Africa is a cause for concern, as the wasp has been recognized as a good natural enemy in comparison to the agents that were sampled from the native range (Wheeler et al. 2001; Hight et al. 2002). Furthermore, given that seedling, survival is low for *S. terebinthifolia* (Ewel 1986); wasp damage may contribute significantly to reducing the spread of *S. terebinthifolia* (Hight et al. 2002). Therefore, determining the extent to which seed predation reduces the invasive potential of *S. terebinthifolia* was important to know if it is reducing its further spread (Milton et al. 2007; Iponga et al. 2008). The low levels of damage in South Africa suggest that there is little impact from the wasp compared to the USA and that the plant is likely to increase in density and distribution via seeds in the absence of further management. It is therefore suggested that biological control be considered for *S. terebinthifolia* in South Africa and that seed-feeding agents should be considered despite the presence of the seed-attacking wasp.

3 Chapter 3: Determining genetic diversity and the introduction history of *Schinus terebinthifolia* in South Africa.

3.1 Introduction

Management of biological invasions can be assisted by knowing the invasion history of a species (Gaskin et al. 2011; Hopper et al. 2018). Genetic analyses are often the only way to elucidate invasion history, as records on the timing and location of introductions are usually lacking or misleading (Estoup & Guillemaud 2010). Phylogeographic studies of introduced species can also give important insights into the number of independent introductions and their subsequent expansion and patterns of gene flow (Shirk et al. 2014). This information can play a crucial role in providing information for developing management strategies for invasive alien species, especially for the development of weed biological control programmes (Goolsby et al. 2006; Paterson et al. 2009; Rollins et al. 2009). Genetic studies to elucidate the invasion history and the source of invasive alien plant populations have therefore become standard practice in biological control programmes (Gaskin et al. 2005; Goolsby et al. 2006; Madeira et al. 2007; Haufbauer & Sforza 2008; Paterson et al. 2009; Paterson & Zachariades 2013; Canavan et al. 2017; Kwong et al. 2017).

In their introduced range, exotic species are expected to have lower genetic diversity because of the limited number of propagules that are typically introduced to found the population (Burdon & Marshall 1981; Williams et al. 2005). There are, however, some cases where genetic diversity of introduced plant populations has been greater than expected, possibly due to multiple introductions and the mode of reproduction of the plant in question (Sutton et al. 2017; Kwong et al. 2017). Plants with clonal modes of reproduction are likely to have lower genetic diversity, such as the apomictically reproducing pompom weed, *Campuloclinium macrocephalum* (Less.) DC (Asteraceae) (Gitonge et al. 2015), and the cactus, *Pereskia aculeata* (Miller) (Cactaceae), which reproduces primarily through fragments of stems broken off the main plant (Paterson et al. 2009), which both had very low levels of genetic diversity in their introduced distributions compared with their native ranges. There are also a number of examples of invasive species displaying rapid evolutionary changes after introduction, which implies that there has been sufficient genetic diversity for selection to act on (Lee 2002; Allendorf & Lundquist 2003; Stockwell et al. 2003). One explanation for this is that some alien plants have had a recovery of genetic diversity, often as a result of multiple introductions from different source populations followed by hybridisation between them (Williams et al. 2005;

2007). A combination of multiple introductions and outcrossing through sexual reproduction is thought to have resulted in the very high levels of genetic diversity of the invasive tree *Spathodea campanulata* (Beuv.) (Bignoniaceae). This is an African tree that has become problematic in the Pacific Islands (Sutton et al. 2017). The high levels of genetic diversity, as well as the sources of the many possible introductions to the Pacific region, are also likely to influence the efficacy of biological control, and knowledge of the invasion history of *S. campanulata* in the Pacific has been an essential component of the biological control programme (Sutton et al. 2017).

Similarly to *S. campanulata,* invasive alien populations of *S. terebinthifolia* in Florida, USA, originated from multiple introductions, resulting in increased genetic diversity and increased invasive potential (Williams et al. 2005; 2007; Geiger et al. 2011). Phylogeographic studies of Florida's historical records of *S. terebinthifolia* have revealed two separate introductions (Morton 1978) that have been found to originate from different geographic source areas in the plants native range in Brazil and are referred to as haplotype A and B in the literature (Williams et al. 2005). Intraspecific hybridisation between the two haplotypes has subsequently occurred and resulted in novel hybrid genotypes (Williams et al. 2005). This has increased genetic variability in the invaded range, and facilitated rapid adaptations to new niches (Williams et al. 2007; Suarez & Tsutsui 2008; Geiger et al. 2011).

Phylogeographic studies also help provide important baseline information for biological control, as herbivores are often sensitive to plant genotype (Lambert & Casagrande 2007; Bhattarai 2015; Cronin et al. 2016). Goolsby et al. (2003; 2006) demonstrated this sensitivity with populations of an eriophyid mite collected for biological control of *Lygodium microphyllum* (Cav.) R. Br. (Lygodiaceae). Mites collected from plants from the source of the invasive population performed better on the weed genotype and had reduced fitness and efficacy on plant genotypes from other parts of the plants native distribution (Goolsby et al. 2003; 2006). In a study on *S. terebinthifolia* in Florida it was shown that the introduced seed parasitoid, *Megastigmus transvaalensis* (Hussey) (Torymidae), performed poorly on the hybrid *S. terebinthifolia* individuals in comparison to the two progenitors (haplotypes A and B) (Geiger et al. 2011). Genetic techniques can assist in determining the source of invasive plant populations, as well as the invasion history, thereby ensuring that an agent is selected from the source area in the native distribution of the introduced species, which is where the most effective agents are likely to be found (Roderick 2004; Goolsby et al. 2006; Gaskin et al. 2011).

The history and pathways of introduction of *S. terebinthifolia* in South Africa are unknown and with the species already a problematic invasive it is important to investigate the extent of genetic variation and to trace the source area(s) in the native distribution. This is especially important for *S. terebinthifolia* because extensive research in the USA has indicated that multiple haplotypes were introduced in that country, that hybridization between haplotypes has occurred, and that this complex invasion history has implications for management and biological control in particular (Callaway & Ascheborg 2000; Levine 2000; Daehler 2003).

This study investigated the invasion history of *S. terebinthifolia* in South Africa using cpDNA sequencing and microsatellites. These techniques are the same as those used in the USA (Williams et al. 2002; 2005), and so are directly comparable. The data were used to determine the source of the South Africa populations of *S. terebinthifolia*, to determine whether a single or multiple introductions of the species have occurred, and to investigate the genetic diversity of the species in the country. This will contribute towards the selection of the most effective biological control agent for *S. terebinthifolia* in South Africa.

3.2 Materials and methods

3.2.1 Sampling and DNA extraction

Fifty-one individual trees were sampled from across the plant's distribution in South Africa (Table 3.1, Figure 3.1). A further eight samples were included from the native (South America) and introduced ranges (Florida and Hawaii, USA - samples 41-48) (Table 3.1). Fresh leaves were dried in silica gel according to the protocol of Chase & Hills (1991). DNA was extracted using the Qiagen DNeasy® Plant Mini Kit (Valencia, CA) following the manufacturer's protocol. The protocol was adjusted in that leaf tissue was crushed under liquid nitrogen prior to the extraction.

Table 3.1 Sampling sites for the collection of *Schinus terebinthifolia* genetic material.

Sample Number	**Site Location**	**Province (in South Africa)**	**GPS coordinates (Latitude; longitude)**	
1A	Shakakraal	KwaZulu-Natal	-29,4443	31,23997
2A	Shakakraal	KwaZulu-Natal	-29,4524	31,21672
3A	R102	KwaZulu-Natal	-29,5048	31,18503
4A	Ballito entrance	KwaZulu-Natal	-29,5041	31,1944
4	Umzinto	KwaZulu-Natal	-30,1261	30,84441
5A	Eshowe	KwaZulu-Natal	-29,0121	31, 57952
5	Hibberdene	KwaZulu-Natal	-30,5704	30,56809
6	Eshowe2	KwaZulu-Natal	-29,0121	31,57952
12	Dube village	KwaZulu-Natal	-29,412	31,27277
14	Shibumi	KwaZulu-Natal	-30,6956	30,41586
17A	Amazimtoti	KwaZulu-Natal	-29,9855	30,93678
18	Port Edward	KwaZulu-Natal	-30,9659	30, 21387
19A	Scottburgh 2	KwaZulu-Natal	-29,9853	30,93679
19	R66	KwaZulu-Natal	-29,0166	31,58311
20	N2	KwaZulu-Natal	-30,0738	30,86943
25	Bendigo	KwaZulu-Natal	-30,6762	30,50448
26	Cnr of Nelson	KwaZulu-Natal	-31,0518	30,20742
31	Nyalaza	KwaZulu-Natal	-28,2143	32, 30701
32	Park Rynie	KwaZulu-Natal	-30,3039	30,66676
33	Mthunzini	KwaZulu-Natal	-29,0202	31,59032
34	N3-Pietermaritzburg	KwaZulu-Natal	-29.80529	30,74637
35	Salt Rock	KwaZulu-Natal	-29,4786	31,21786
36	Munster	KwaZulu-Natal	-31,0017	30,24848
37	R61	KwaZulu-Natal	-31,0017	30,24849
38	Umzinto DR	KwaZulu-Natal	-30,1261	30,84441
39	Durban rail reserve	KwaZulu-Natal	-29,5294	31,16971

40	Wild coast	KwaZulu-Natal	-31,084	30,18862
1	Onrus	Western Cape	-32,3186	18,40576
13	Magnolias	Western Cape	-33,9449	18,84668
15	Onrus2	Western Cape	-34,4071	19,16236
16A	Napier	Western Cape	-32,3186	18,40576
2	Biscay rd	Eastern Cape	-33,5314	26,88238
3	South down	Eastern Cape	-33,6165	26,87168
7	Word of truth	Eastern Cape	-33,5871	26,90543
11	Willshire	Eastern Cape	-33,3055	26,50949
11A	Cause way	Eastern Cape	-33,5938	26,88705
27	Strand st	Eastern Cape	-33,5897	26,90413
28	Cause way 2	Eastern Cape	-33,5938	26,88687
29	Word of tructh2	Eastern Cape	-33,5897	26,90408
30	West rd	Eastern Cape	-33,5911	26,88248
6A	N2 close to M65	Eastern Cape	-29,6261	31,12441
7A	Wilshire 1	Eastern Cape	-33,3168	26,52654
8A	Market st2	Eastern Cape	-33,3146	26,53056
9A	Currie st	Eastern Cape	-33,3021	26,53455
10A	York St	Eastern Cape	-33,3138	26,5369
12A	Willshire2	Eastern Cape	-33,3051	26,50857
13A	Plumbridge dr	Eastern Cape	-33,2926	26,52554
17	Bhisho main rd	Eastern Cape	-32,8586	27,43221
21	Highfield	Eastern Cape	-32,9608	27,9354
22	Symtheland rd	Eastern Cape	-32,9549	27,90663
23	Kenton RD	Eastern Cape	-33,6024	26,85664
41	BZGW15	Ubatuba, State of São Paulo	-23,45876	-45,0688
42	HW9	Hawaii- Big island (Kula)	20,796538	156,326537
43	SP22	SãoSebastião, State of São Paulo,	-23,78708	-45,56731
44	GUR1(2)	Pontal do Paraná, State of Paraná.	-25,62817	-48,4795
45	IDN4	Fellsmere, Florida	27,76718	-80,53387

46	RS21	Santo Antônio das Missões, RS	-28,46894	-55,15503
47	SC15	Itapoá, State of Santa Catarina	-26,06715	-48,60785
48	SC22	Dezesseis de Novembro, São Joaquim - State of Santa Catarina	-28,20474	-49,9869

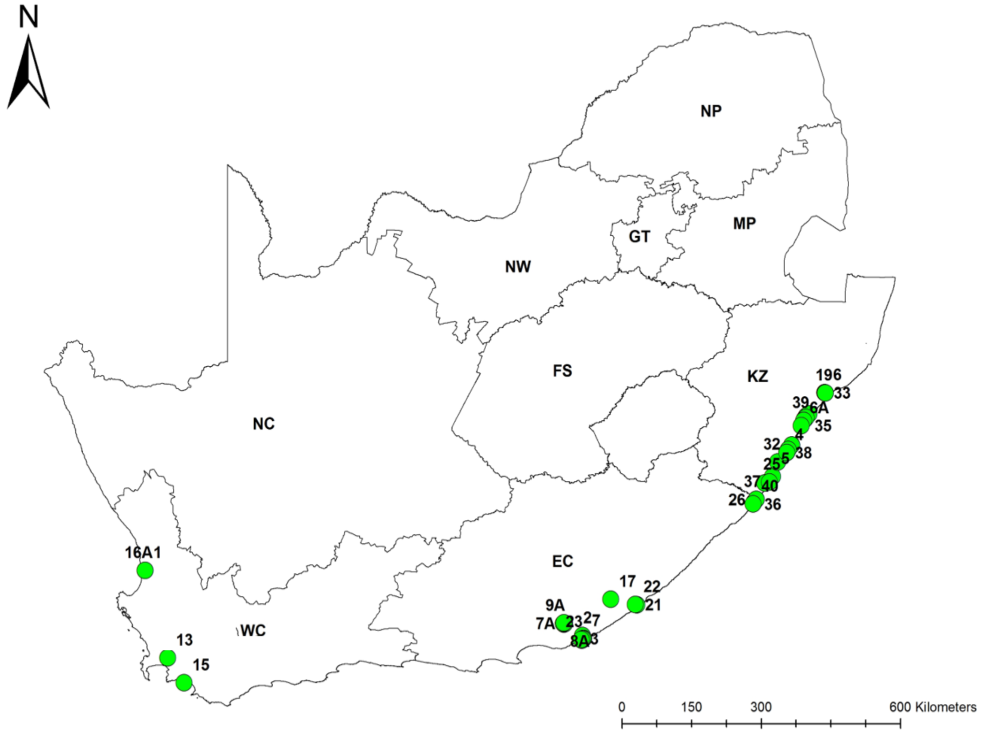


Figure 3.1 Map of South Africa showing distribution of sampled populations of *S. terebinthifolia*, with corresponding sample numbers (Table 3.1).

3.2.2 Haplotype analysis using Chloroplast DNA

This method was derived from Williams et al. (2005) and Hamilton (1999). Chloroplast DNA (cpDNA) intraspecific variation was assessed by amplifying part of the *Trn* intergenic spacer regions using the primers *trnS* and *trnG* (Table 3.2). The Polymerase chain reason (PCR) reaction contained 0.4 µl of each of the primers, 10 µl of Promega Master Mix (Madison, WI), 0.8 µl of Promega MgCl 2, 3 µl of template DNA per reaction. A further 5.4 µl Promega nuclease-free water to reach a final volume of 20µl. Amplifications were performed in a T100 ™ thermal cycler (Bio-Rad. South Africa). The PCR protocol had a 5 min denaturing step at 96°C followed by 40 cycles of 96 °C for 45s, annealing temperature of 52°C for 1 min, and 72 °C extension for 30s, as in Hamilton (1999). PCR products were sequenced at Macrogen Corp., Korea using a DNA Analyser 3730x (Applied Biosystems™, Foster City, CA). Samples received from the native range and Florida had already had their haplotypes determined and reported in (Williams et al. 2005). The cpDNA analysis was therefore only conducted on South African samples.

Table 3.2 Primer sequences used to obtain *S. terebinthifolia* haplotypes (from Hamilton 1999).

Primer	**Sequence(5’-3’)**	**Length of obtained sequence** (bp)
trnS	GCCGCTTAGTCCACTCAGC	844
trnG	GAACGAATCACACTTTTACCAC	844

3.2.3 Microsatellite analysis

The six *S. terebinthifolia* microsatellite loci were grouped into two multiplex reactions according to the protocols of Williams et al. (2002) and Williams et al. (2005) (Table 3.3). Applied Biosystems™ added fluorescent labels to the primers. The PCR contained ±10 ng of DNA, 12.5 µl of Q5 High-Fidelity 2X Master Mix, and 2.5 uL of each primer at concentrations of 10 uM to make a total volume of 25 µl. Reactions were cycled in a Mastercycler® nexus (Eppendorf, Germany), 230 V/50 – 60 Hz using the simulated tube function. Cycling parameters were one cycle at 98 °C for 30s, followed by 35 cycles of 10s at 98°C, 20s at 50°C (multiplex A) or 55 °C (multiplex B), 20s at 72 °C, and a final cycle at 72 °C for 2 min. Capillary electrophoresis were run on an ABI 3500XL genetic analyser at Inqaba Biotec ™.

Microsatellite results can contain errors due to allelic dropout or false allele amplification and this will influence allele frequency estimates and identification of genotypes (Bonin et al. 2004). To address this error, 10% of samples were duplicated and the results were compared.

To avoid subjectivity in scoring of peaks, any peaks that were ambiguous were scored as missing data.

Table 3.3 Primer sequences used in this study and their allelic diversity measures for six microsatellite loci of *S. terebinthifolia* from Williams et al. (2002) and Williams et al. (2005).

Locus	**Primer sequence (5'-3')**	**Fluorescent Dye**	**Allele size range (bp)**
StAAG13	F:TCACGTTCATGATGCAAAGA	6-FAM	162-224
	R: TGGGTAGGTGATGCAGTTCTA		
	R: GATTCCCACGTCAGATTCGT		
StAAG14	F:GCAGAATCACACTACTCAGTCACC	VIC	157-202
	R: TGGAATGGGTTGGAGGTAGA		
StGGT39F	F:GACACACCCAAATGACTCACA	NED	200-258
	R: CGGGCAGAATTTGATGAAGA		
StCTCCTT01	F:TCTCGCATTTCAAGATCACG	PET	126-176
	R: AGCAGTAGGTCGAGGAGGAGT		
StAAT1	F:AAGGGTGAGAATCTGAAATTTA	6-FAM	114-192
	R: GGCAAACCATTAGTGAGTTTAT		
StAAT16	F:AACAGCCCACCATTTTAACA	NED	152-213

3.3 Statistical analysis

Chloroplast DNA chromatograms were examined, and contiguous sequences were assembled and manually edited in ChromasLite™ ver. 2.6.4. An alignment of sequences was done in GeneStudio™ version 2.2.0.0 and included all cpDNA haplotypes for *S. terebinthifolia* that were downloaded from GenBank (Accession numbers: AY928398-AY928407) with haplotypes confirmed by visual inspection.

For the microsatellite data, chromatogram alignment was first constructed using Geneious ver. 11.1.5 (Kearse et al. 2012). Peak size markers were all aligned to ensure amplified peaks could be aligned by fragment size. The dataset was entered into a co-dominant matrix and analysed using GenAIEx ver. 6.5 (Peakall & Smouse 2012). Pairwise genetic distances were calculated based on the number of shared alleles per locus (Euclidean distances). The output matrix of genetic distances was then used to run a Principal Component Analysis. Allelic diversity was measured as the total number of alleles per locus. Genetic diversity was compared between populations by calculating Nei's unbiased genetic identities (Nei 1973), number of effective alleles (Ne) and the Shannon Information Index (I) (Lewontin 1995), with the program PopGene ver. 1.32 Population Genetic Analysis (Yeh & Boyle 1997).

The sample haplotypes were determined based on their cpDNA matrilineages. The nuclear ancestry of the South African and native populations were subsequently tested based on the six microsatellite loci using a Bayesian genetic clustering algorithm implemented in STRUCTURE version 2.3.4. (Pritchard et al. 2000). An admixture model was used that assumed correlated allele frequencies with 10 iterations for each run. Each run consisted of 1,000,000 MCMC steps and a burn-in period of 100,000. The number of populations (K) was tested from 1-10 and K was inferred with Harvester (following Evanno et al. 2005). Models were also run including only South African samples according to the same parameters. Membership assignment of each population was estimated as (*q*), the ancestry coefficient, which varies on a scale from 0 to 1.0, with 1.0 indicating full ancestry with a certain a population.

3.4 Results

3.4.1 Chloroplast DNA

All 51 trees sampled in South Africa had identical cpDNA sequences. These sequences were a 100% match to *S. terebinthifolia* haplotype A samples from previous studies (Williams et al. 2005; 2007) (Genebank Accession number for haplotype A-J: AY928398-) (Table 3.4)

Table 3.4 Description of cpDNA haplotypes, indicating polymorphic sites and their position to base pairs indicating the similarities of South Africa samples with haplotype A. Sequences of *Schinus terebinthifolia* from GenBank (Accession nos AY928398–AY928407) (Williams et al. 2005).

Regions	**haplotype**	**Position Base Pairs**												
		11	204	219	225	227	244	354	384	447	552	582	657	676
South Africa	A	T	A_{11}	G	T	-	C	A	A	T	A	T	T	C
Brazil	A	T	A_{11}	G	T	-	C	A	A	T	A	T	T	C
	B	G	A_{14}	G	G	-	C	A	A	C	A	T	T	C
	C	T	A_{14}	G	T	-	A	T	A	C	C	G	T	T
	D	T	A_{13}	G	T	-	A	T	A	C	C	G	T	T
	E	T	A_{14}	C	T	-	A	T	A	C	C	G	T	T
	F	T	A_{12}	G	T	-	A	T	A	C	C	T	T	C
	G	T	A_{11}	G	T	-	A	T	A	C	C	T	T	C
	H	T	A_{15}	G	T	-	A	T	A	C	C	G	T	T
	I	T	A_{14}	G	T	C	A	T	-	C	C	G	T	T
	J	T	A_{12}	G	T	-	A	T	A	C	C	T	G	C

3.4.2 Microsatellite analysis

An overall error rate of 36.1% was found in the microsatellite analysis, based on replication of 10% of the samples. The overall error was predominantly a result of allelic dropout and false allele amplification in samples for primers. stAAG13 (67%) and stAAG14 (50). Error rates for each of the other primers was; 25% for StGGT39F; 17% for StCTCCTT01; 25% for StAAT1 lastly 33% for StAAT16.

All but three of the samples from outside of South Africa grouped together but besides that group, there was very little evidence of structuring according to geographic location in the PCoA (Figure. 3.2). Samples 43, 44, 45, 46 and 48, all from the indigenous distribution in South America or the introduced distribution in Florida, USA, formed a group (Figure. 3.2). Samples 41 and 47, from both South America, and sample 42 from the introduced distribution of Hawaii, USA, were mixed with samples from South Africa (Figure. 3.2). Within the South African samples, there was no geographic pattern in allelic phenotypes as no clear clustering was found according to geographic area. Some samples shared the same allelic profiles, such as samples 12A and 31; samples 1 and 3A; samples 5A, 6A and 10A; as well as samples 36 and 37.

Based on their microsatellite phenotypes, STRUCTURE HARVESTER analysis suggested that the optimal number of clusters was two (k = 2) (Figure. 3.3. & 3.4). When running the analysis with two populations (i.e.: k =2), sample 42 (from Hawaii) was grouped in the same population as the South African material while all other samples from outside of South Africa, including samples 41 and 47 which clustered with South African samples in the PCoA, fell into a second population. The sample from Hawaii (sample 42) also grouped with South African samples in the PCoA and shared the same alleles as South African samples as a result of a shared ancestry (q = 0.995 average proportion of ancestry with South African populations).

The separate STRUCTURE analysis of only South African samples grouped all the samples into three populations (k = 3) (Figure. 3.5 and 3.6). STRUCTURE analysis, as with the PCoA analysis, found no geographical pattern among these populations.

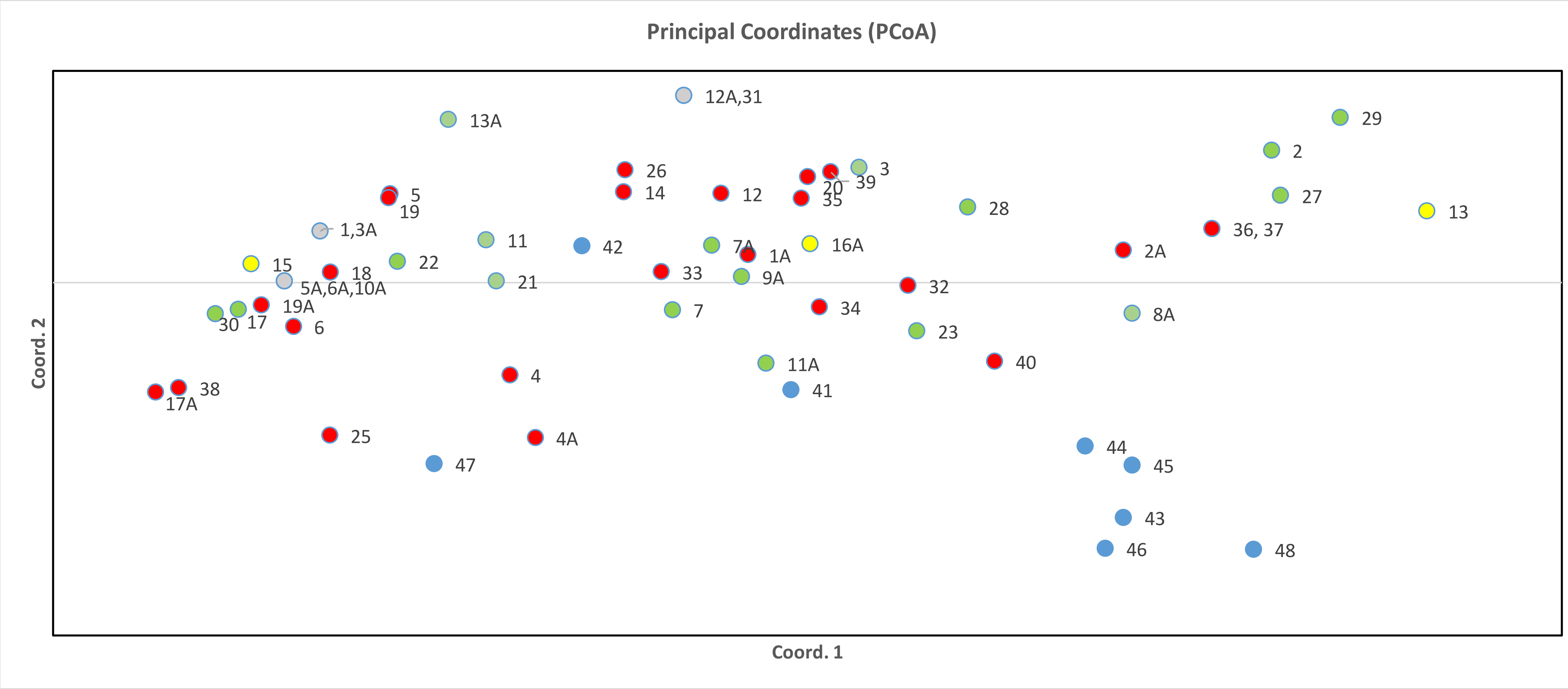


Figure 3.2 Microsatellite diversity in *Schinus terebinthifolia* samples in South Africa (samples 1A-40) and the native and other introduced ranges (samples 41-48). The primers were stAAG13, stAAG14, stGGT39F, stCTCCTT01, stAAT1 and stAAT16. Coordinate 1 accounts for 19% and Coordinate 2 accounts for 16% of the variation. Colours represent origins of the plants. Red = KwaZulu-Natal Province, green = Eastern Cape Province, yellow = Western Cape Province, grey- mixed geographic areas, blue = native range in South America and introduced range in Florida

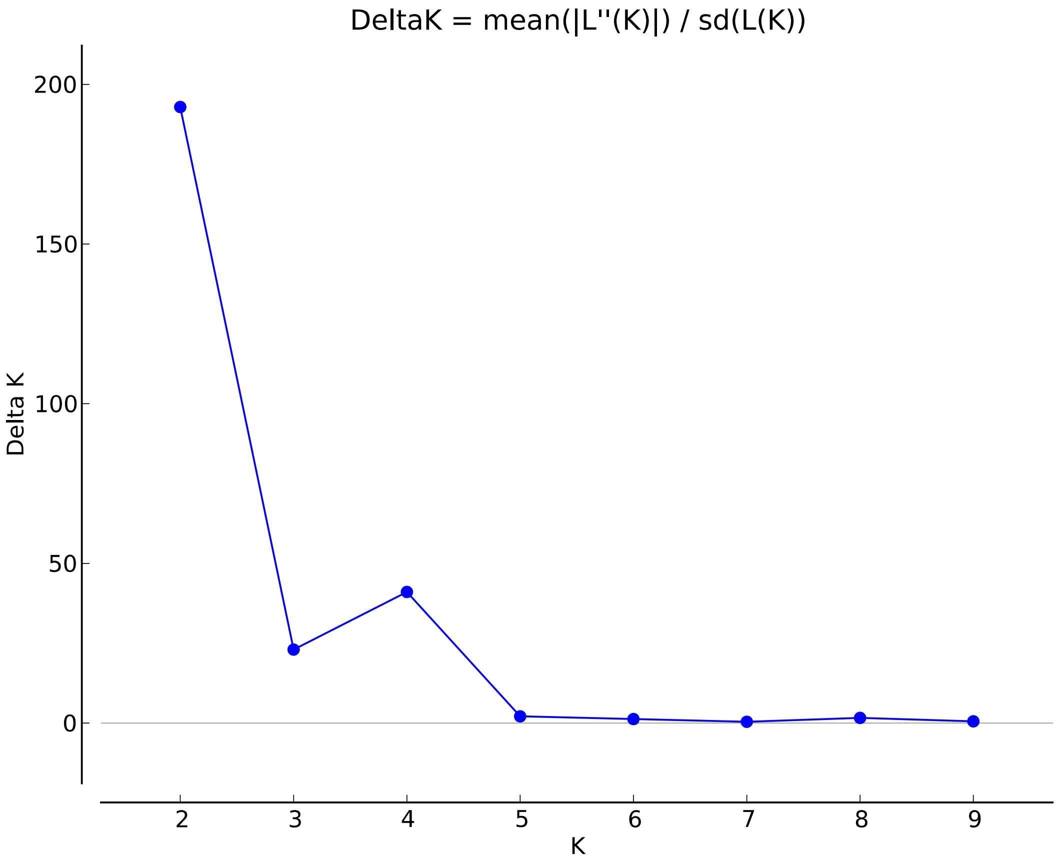


Figure 3.3 Graph of Delta K values showing the ideal number of populations as *k* = 2 based on 59 samples of *Schinus terebinthifolia* from South Africa and the native and introduced range (when including populations), using 6 microsatellite primer pairs and the Evanno method implemented in STRUCTURE HARVESTER program according to Earl and von Holdt (2012).

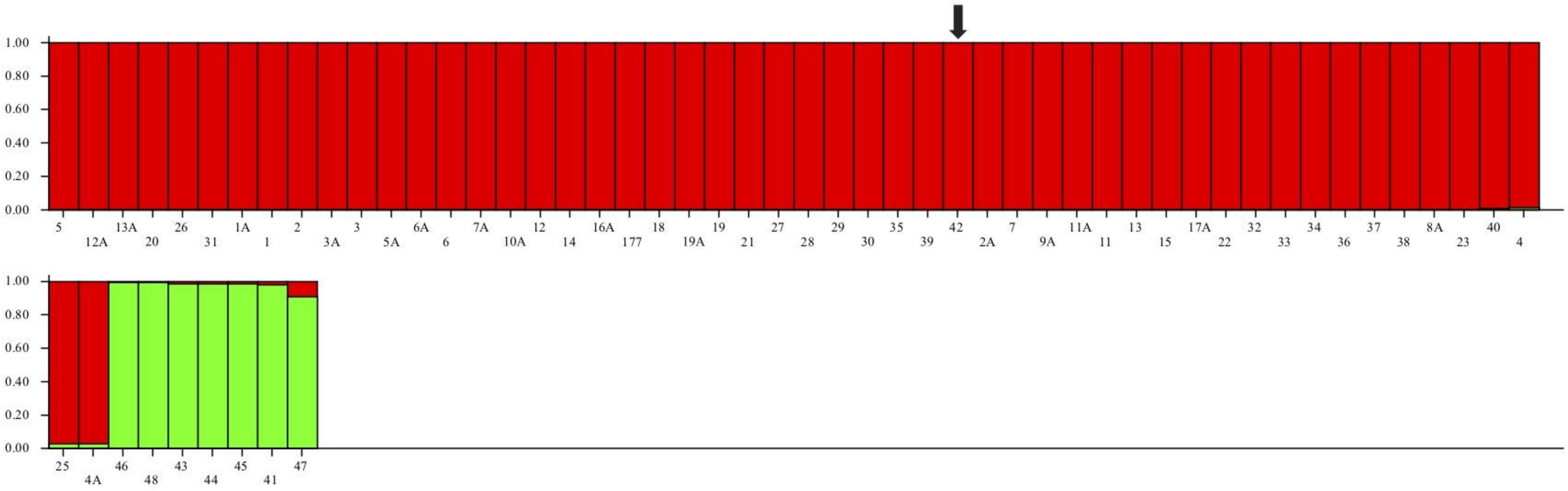


Figure 3.4 Genetic population structure of 59 individuals of *Schinus terebinthifolia* from populations in South Africa, the native range in South America and the introduced range in Florida, based on Bayesian clustering analysis of 6 microsatellite loci with STRUCTURE (Pritchard et al. 2000). According to the Evanno method, two populations were inferred (Figure 3.3). Samples are ordered according to their Q values. The red cluster corresponds to South African populations and the green cluster represents populations from the native range. The values in ordinate the shared ancestry according to percentage membership into each population. The downward arrow points to sample 42 from Hawaii, USA.

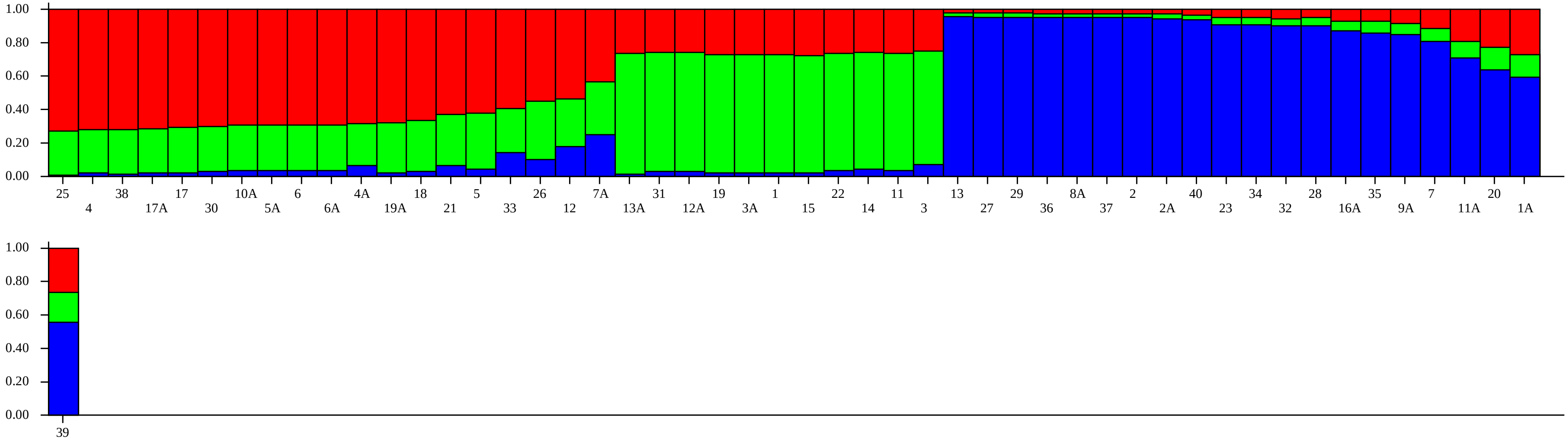


Figure 3.5 Genetic population structure of 51 individuals of *Schinus terebinthifolia* from populations in South Africa, based on Bayesian clustering analysis of 6 microsatellite loci with STRUCTURE (Pritchard et al. 2000). According to the Evanno method, three populations were inferred (Figure 3.6). Sample ordered according to Q value. The red cluster corresponds to population 1, the green cluster corresponds to green and blue corresponds to population 3. The values in ordinate the shared ancestry according to percentage membership into each population.

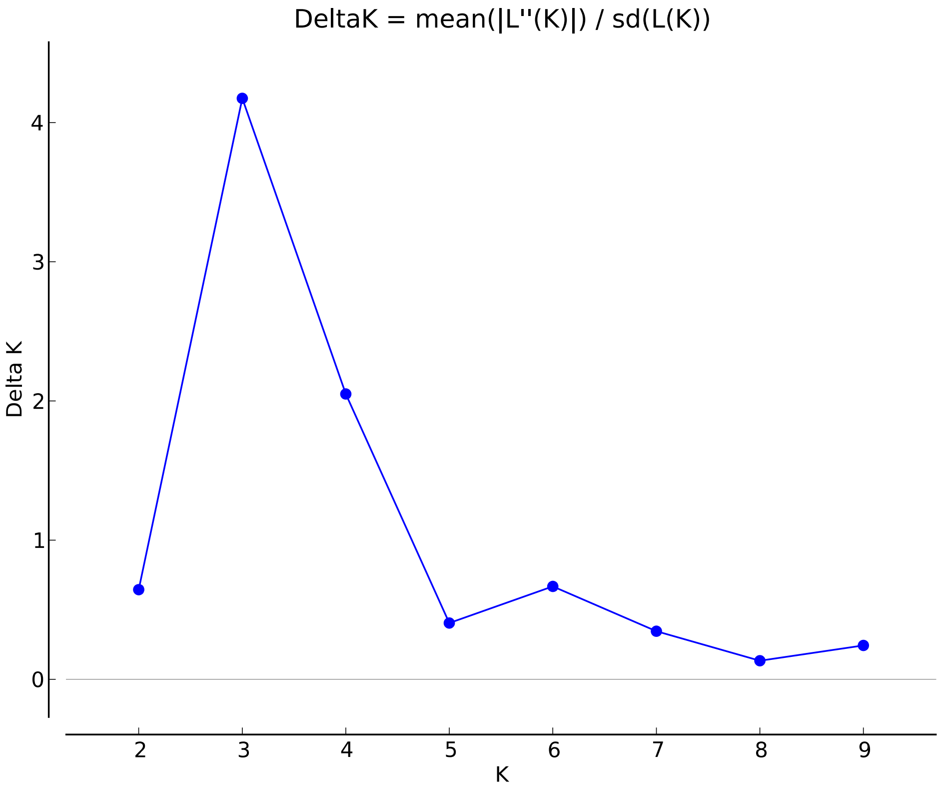


Figure 3.6 Graph of Delta K values showing the ideal number of populations as *k* = 3 based on 51 samples of *Schinus terebinthifolia* from South Africa using six microsatellite primer pairs and the Evanno method implemented in STRUCTURE HARVESTER program according to Earl and von Holdt (2012).

Population 2; which contains all samples (except sample 42) from the native range and Florida was found to have higher genetic diversity in the Shannon Information Index (I), number of effective alleles (Ne) and higher Nei's diversity (h) compared to samples in population 1 (containing South African samples and a sample from Hawaii) (Table 3.5). However, conclusive comparisons of genetic diversity, more samples are needed from the native range. The South African samples (Population 1) were found to have a lower mean number of alleles ($N_{A\,=}$ 2.33), compared to the native range ($N_{A\,=}$ 11.8), Florida ($N_{A\,=}$ 4.4) and Population 2 (N_A =5.50). The Hawaii population according to Williams et al. (2005) had the lowest number of alleles (N_A= 1.4). Population 1 is mostly homozygous (mean H_O = 0.29) whereas populations 2, Florida and South America are mostly heterozygotes (H_0 = 0.43, H_0 = 0.62, and H_0 = 0.39

respectively) (Williams et al. 2005). The Hawaii population was found to be almost completely homozygous ($H_0 = 0.07$) (Williams et al. 2005). South African *S. terebinthifolia* populations shared a number of dominant alleles with South American and Florida populations, for example for primers *stGGT39F* and *stCTCCTT01* samples from both the native and introduced range shared the same dominant alleles (Table 3.6).

Table 3.5 Comparison of genetic diversity for populations 1 (South African and native range sample 42) and population 2 (native range and Florida samples) based on six microsatellite primer pairs (over populations for each loci). Sample size (N), mean number of observed number of alleles (N_A), expected heterozygosity (H_E), observed heterozygosity (H_O), number of effective alleles (Ne), Shannon Information Index (I), Nei's gene diversity (h), and standard deviation (s.d.). Samples from the native range (South America) and another invaded ranges (Florida and Hawaii) (Williams et al. 2005) were included for comparison. The values borrowed from Williams et al. (2005) are based on five microsatellite loci.

	N	**N_A (s.d.)**	**H_E (s.d.)**	**Ho (s.d.)**	**Ne (s.d.)**	**I (s.d.)**	**H (s.d.)**
Pop 1	52	2.33±1.03	0.41±0.25	0.29±0.22	1.97±0.89	0.65±0.43	0.41±0.24
Pop 2	7	5.50±1.38	0.74±0.09	0.43±0.18	4.21±1.38	1.51±0.31	0.73±0.09
Hawaii	15	1.4	0.09	0.07			
Florida	354	4.4	0.58	0.62			
South America	108	11.8	0.46	0.39			

Table 3.6 Genetic characteristics of the six microsatellite loci genotyped comparing the two populations from this study. *N*: Number of samples genotyped; N_A Number of alleles; H_E expected heterozygosity; H_O observed heterozygosity. Dominant phenotypes: dominant allele phenotypes, values in parentheses are the frequency of each phenotype (- denotes when there are no shared phenotypes within the population).

Locus	Pop 1 (South Africa and sample 42)	Pop 2 (native range and Florida)
stAAG13		
N	52	7
N_A	2	7
H_E	0.44	0.87
H_O	0.38	0.57
Dominant phenotype	180/180 (0.48)	
stAAG14		
N	52	7
N_A	2	6
H_E	0.50	0.86
H_O	0.48	0.71
Dominant phenotype	176/182 (0.48)	161 (0.29)
stGGT39F		
N	52	7
N_A	4	5
H_E	0.73	0.69
H_O	0.15	0.28
Dominant phenotype	197 (0.25)	197(0.43)
stCTCCTT01		
N	52	7
N_A	2	5
H_E	0.50	0.69
H_O	0.56	0.28
Dominant phenotype	143/155 (0.56)	155(0.43)

stAAT1		
N	52	7
N_A	3	4
H_E	0.30	0.71
H_O	0.85	0.29
Dominant phenotype	125 (0.75)	122 (0.29), 125 (0.29)
stAAT16		
N	52	7
N_A	1	7
H_E	0	0.90
H_O	0	0.43
Dominant phenotype	147 (1)	

3.5 Discussion

Both microsatellite and cpDNA analysis of *S. terebinthifolia* sampled in South Africa indicate that only haplotype A was present. If other haplotypes are present in the country, then they are either found in isolated populations that were not sampled, or are very rare. The majority of invasive *S. terebinthifolia* that requires management in South Africa are therefore haplotype A. In the native distribution, haplotype A is found in Balneário Camboriú and Santa Catarina, in Brazil (Williams et al. 2005). In South America, ten haplotypes of *S. terebinthifolia* have been recorded however, haplotype A has been found to be the most common and has been recorded in all the invaded regions of Hawaii, Florida, Texas, US Virgin Island (Williams et al. 2005) and now South Africa.

All South African populations have similar allelic phenotypes with two samples from the native range and are a very close match to the sample from the introduced range in Hawaii. Furthermore, sample 42 from Hawaii was found to belong to the same population as South African samples according to STRUCTURE, indicating shared ancestry. In both South Africa and Hawaii, haplotype A is the recorded cpDNA haplotype (Williams et al. 2005). Both these regions are regions where *S. terebinthifolia* is an introduced tree mostly for ornamental purposes suggesting that the plant in both these regions came from the horticulture industry. *Schinus terebinthifolia* in South Africa is therefore likely to have originated from a single introduction from Hawaii, USA, or from the same source as those in Hawaii in the horticultural industry. It is also possible that *S. terebinthifolia* was introduced from the horticultural industry into South Africa and was then spread to Hawaii. Invasive alien plants are often introduced through the horticultural trade and evidence of hybridization and artificial-selection as a horticultural plant has been recorded for other serious invasive alien plants such as *Lantana camara* L. (Verbenaceae) (Spies & Striton 1982) and *P. aculeata* (Paterson et al. 2009). Plants that have been changed through hybridization and artificial-selection may be more difficult to control using biological control as they may be different from the plants in the native range where natural enemies would have evolved (Paterson et al. 2009; Urban et al. 2011).

Williams et al. (2005) found populations of *S. terebinthifolia* in the native range to be structured according to geographic distance with strong subdivision of haplotypes because of limited seed dispersal. However, in South Africa there was no geographical population structure found. Unlike in the native range, humans have moved *S. terebinthifolia* across South Africa for ornamental, hedging and shade/shelter purposes (Panetta & McKee 1997). In the Eastern Cape *S. terebinthifolia* was planted as a street tree in Grahamstown, Port Alfred and Somerset East (Kuruneri-Chitepo & Shackleton 2011). In KwaZulu-Natal, farmers used the trees for boundaries and wind breaks (Jones 2018). *Schinus terebinthifolia* is a prolific seed producer (Geiger et al. 2011) and seeds can be distributed over long distances via birds. Native frugivorous birds play an important role in dispersal and germination of seeds (Dlamini et al. 2018). They help reduce germination time and improve success through pulp removal (Dlamini et al. 2018). Therefore, the presence of three populations in South Africa with no geographic structuring is likely a result of both human movement of the trees and native frugivorous birds spreading the seeds over long distances.

The South Africa population was found to have samples that remained genetically similar to populations from the native range (samples 41 and 47) with a number of shared dominant phenotypes. This indicates a close connection with the native range and with only a single haplotype in South Africa, there is no evidence of the hybridisation that occurred in Florida. Looking at the allelic diversity of South African populations there is evidence that genetic diversity is low when compared to the other introduced ranges as well as the native range. Genetic diversity of alleles in alien plants has generally been found to be low due to populations being founded by just a few individuals from the native range (Sakai et al. 2001). South African populations were also found to have reduced heterozygosity compared to both the native range in Brazil and invaded range in Florida. Loss of heterozygosity is known to limit a population's ability to respond rapidly to selection after a bottleneck (Maruyama & Fuerst 1985).

Studies of *S. terebinthifolia* in its invasive range have found that even when the plant has reduced genetic diversity this often does not limit its invasive potential. For example, in Hawaii where populations have the same haplotype and low allelic diversity, the plant is one of the most significant non-indigenous species currently threatening federally listed endangered native plants (Hight et al. 2002). This coincides with a study on *Hypericum canariense* L. (Hypericaceae) which demonstrated that even isolated founding populations (organisms that have little genetic mixing with other organisms within the same species) may still quickly adapt (Dlugosch & Parker 2008) and can evolve novel invasive behaviour (Stockwell et al. 2003; Stockwell & Ashley 2004). The success of *S. terebinthifolia* seems to be favoured by the susceptibility of the invaded areas due to having under-utilised niches, low species richness and increased human disturbance regimes (Callaway & Ascheborg 2000; Levine 2000; Daehler 2003).

Williams et al. (2005) suggested that the population in Hawaii was introduced from Florida based on the presence of monomorphic alleles. The low allelic diversity in South Africa as well as Hawaii makes managing the plants using biological control easier than cases where there is high genetic diversity. The genetic similarities between South Africa and Hawaii population should potentially mean that biological control agents that are successful in Hawaii are likely to be successful in South Africa and vice versa. This is not the case in North America, where hybridisation has resulted in novel genotypes (Geiger et al. 2011). This evidence of only one source population is positive news for a biological control programme for *S. terebinthifolia* as invasive populations with low genetic diversity are more likely to be controlled successfully through biological control (Paterson et al. 2009).

The results from this study have determined that only one haplotype of *S. terebinthifolia* has been introduced into South Africa. Populations in South Africa were found to have shared genotypes with both samples from the native range and introduced range in Hawaii. This indicates a shared source area with invasive populations in Hawaii. The genetic information on the invaded population in the invaded region is important in assisting with identifying natural enemies. Biological control agents sourced from haplotype A plants in South America, and from sites 41 in Ubatuba, State of São Paulo and 47 in Itapoá, State of Santa Catarina will be particularly good matches for South African *S. terebinthifolia*. Additionally, any biological control agent that is suitable for the genotypes of plants in Hawaii, USA, are likely to be suitable for the South African genotypes. The climatic conditions in Hawaii, as well as in South America, may however be very different from those in South Africa, so matching a suitable climate from which to source biological control agents is likely to be equally as important as matching the most appropriate plant genotype.

4 Chapter 4. The potential distribution of *Schinus terebinthifolia* in South Africa

4.1 Introduction

Predictive modelling of geographic distributions of invasive alien species has many benefits, including identifying regions that are not yet invaded but are climatically suitable and are therefore likely to be invaded in future (Phillips et al. 2006). It has been used in invasive species risk assessments and for the prioritisation of species for management (Leung et al. 2012). In weed biological control, predictive modelling has been used to determine where to search for potential biological control agents that are climatically suited to the invasive distribution and are therefore likely to be effective biological control agents (Trethowan et al. 2011; Paterson et al. 2014; Sutton 2019).

There are two types of predictive modelling, correlative or mechanistic models. Mechanistic models are inherently more complex as they aim to simulate the actual processes that produce the apparent correlations between an organism and an environment, using the known biological limits of a species to predict its distribution (Beerling et al. 1995; Robertson et al. 2003). Mechanistic models are robust in predicting whether a species could occur in a given environment and form the basis of CLIMEX, the most widely used mechanistic software package in invasive species modelling (Sutherst et al. 1999; Magarey et al. 2018). One of the major limits of mechanistic models is the difficulty of obtaining suitable data. If the data is not easily available it can be both time consuming and expensive to obtain. In contrast to mechanistic models, the data required to fit correlative models are widely available and easy to obtain (Elith et al. 2010). Correlative ecological niche modelling is based on an organism's relationship to its environment. It works by using correlations between the known distribution of a species and the environmental parameters of the site or sites where it occurs (Robertson 2004; Magarey et al. 2018). Numerous correlative modelling methods exist and these can be divided into cases where both presence and absence data are available or where just presence data are available (Caithness 1995; Yackulic et al. 2013).

When using presence-only data to make predictions, one can either use a standard profile technique, or opt for a group discrimination technique that makes use of artificially generated pseudo-absence data (Zaniewski et al. 2002). Some of the more prevalent correlative modelling techniques used in invasive alien plants modelling include BIOCLIM (Busby 1991), DOMAIN (Carpenter et al. 1993), factor analysis (Hirzel et al. 2002) and Principal Components Analysis in the program Floramap (Jones & Gladkov 1999). There are several different types of group discrimination techniques, of which the most popular are Generalized Additive Models (GAM) (Austin & Meyers 1996) and Generalized Linear Models (GLM) (Pearce & Ferrier 2000; Guisan & Zimmerman 2000). MaxEnt (Phillips et al. 2006) is regarded as one of the better correlative distribution modelling software packages (Elith et al. 2011; Thompson et al. 2011).

4.1.1 Maximum Entropy (MaxEnt)

MaxEnt is one of the most widely used methods for making predictions or inferences from incomplete information (Phillips et al. 2006). As it has been shown to outperform similar models and has become one of the premier species distribution software packages (Phillips et al. 2017). Since its introduction in 2006, it has been cited in over 6000 publications (Phillips et al. 2017).

MaxEnt software uses a set of input layers, comprising environmental variables (such as temperature and precipitation) transformed into grid cells, as well as a set of georeferenced occurrence locations or ‘training data’ to make predictions (Phillips et al. 2006). Training data (species distribution data) are used to create the predictive model; test data are used to assess accuracy of these models. The model requires data sets to have the exact number of grid cells (geographic bounds) in the input layers so grid cells of the same locality can easily be compared or combined. The model then expresses the suitability of each grid cell as a function of the environmental variables at that grid cell (Phillips et al. 2006). A high value of the function at a particular grid cell indicates that the grid cell is predicted to have suitable conditions for that species (Phillips et al. 2006). The model is then computed as a probability distribution over all the grid cells. The distribution chosen is the one that has maximum entropy (i.e., that is most spread out or closest to uniform) subject to some constraints: it must have the same set of characteristics for each feature (derived from the environmental layers) as the average over sample locations (Phillips et al. 2006). If an area in the study has a similar distribution as the training data, then higher values are assigned, and accordingly, areas with different distributions are assigned lower values (Negga 2007).

4.1.2 Study species

It is regularly stated that models are as good as the data that are used to calibrate them (Fielding & Bell 1997; Hirzel et al. 2001; Wolmarans et al. 2010). Therefore the more studied the species the more data is available to calibrate the models, the better the models. *Schinus terebinthifolia* is regarded as one of the most problematic and widespread invasive species in Florida, USA, covering an area of 3000 km² and is recognised as a noxious weed within the state (Morton 1978; Hight et al. 2002). The importance of the weed means it has been extensively studied for over 50 years (Morton 1978). These studies have included history of introduction and how the species has successfully colonised the USA (Williams et al. 2005; 2007), impacts (Morton 1978), spread, genetics (Mukherjee et al. 2012), biological control (Hight et al. 2002; Wheeler et al. 2016), and finally, the predicted distribution of the species into the USA (Mukherjee et al. 2012). The work done on *S. terebinthifolia* highlighted the intraspecific genetic variation of this species. In Chapter 3, this genetic information is discussed and plants in South Africa were shown to be 'haplotype A'. There is therefore excellent data available with regard to *S. terebinthifolia*. Some of the most important information, other than distribution, is the biology of the species, especially in the invaded range, which can guide the selection of suitable environmental variables and background data that are appropriate to the species in question.

The main aims of this Chapter were to: (1) predict the potential distribution of *S. terebinthifolia* in South Africa using the ecological niche modelling software MaxEnt, and (2) identify suitable areas in the native range where potential biological control agents might be sourced, based on climatic compatibility with the weeds' invaded range in South Africa. Six separate models were created to determine the potential distribution of *S. terebinthifolia* in South Africa, using: (1) invasive range South African occurrence locations; (2) invasive range South African occurrence locations with Koppen Geiger representative backgrounds (Koppen Geiger are climatically representative used for climate classification); (3) invasive range South African and Florida haplotype A occurrence locations; (4) invasive range in South African, Florida and native range haplotype A occurrence locations; (5) invasive range in South African, Florida and native range haplotype A occurrence locations, with a different set of environmental parameters, and; (6) only native range occurrence locations. Finally, a model was created to determine the most suitable native range locations to survey for potential biological control agent based on the invaded range South African occurrence records.

4.2 **Methods and Material**

The MaxEnt software uses a set of georeferenced species occurrence locations or 'training data' as well as a set of input layers, or environmental parameters (such as temperature and precipitation). Once the model is completed the software uses a subset of the occurrence locations 'testing data' to test the models. It is important to normalize the background data to a suitable size to avoid overfitting the models (Van der Wal et al. 2009). These are important components of a MaxEnt model as they determine the accuracy of the outputs.

4.2.1 Species occurrence locations: Native range distribution

These localities were obtained from the Invasive Plant Research Laboratory, USDA/ARS. The methods of native range collection are presented in Williams et al. (2005; 2007) and Mukherjee et al. (2012). Native range species occurrences were recorded in Argentina, Brazil and Paraguay.

4.2.2 Species occurrence locations: Invaded range – USA

In the USA the different haplotypes of *S. terebinthifolia* occupy different environmental niches (Mukherjee et al. 2012). In Florida, USA, two distinct chloroplast haplotypes were found (See Chapter 2; Williams et al. 2005; 2007). Occurrence data for 707 trees distributed throughout the invaded range in Florida were obtained from Department of Biology, Texas Christian University, and Fort Worth, USA. These occurrence data were used in the genetic analysis of Brazilian pepper in the USA, as well as in determining whether the species has undergone a niche shift in the USA (Williams et al. 2005; 2007; Mukherjee et al. 2012). These data were refined to include only the 389 haplotype A occurrence locations from Florida.

4.2.3 Species occurrence locations: Invaded range - South Africa

Occurrence locations in the invaded range of *S. terebinthifolia* were obtained from the Southern African Plant Invaders Atlas (SAPIA) database. The SAPIA database is one of the most comprehensive databases on invasive alien plants in Africa. The majority of these data points are recorded from extensive roadside surveys that have been conducted over the last 20 years by the same researchers. Therefore, they are an excellent indication of *S. terebinthifolia* distribution in South Africa. Additionally, 51 locations from surveys conducted during this study were also added, 136 occurrence locations were used in the model for the invaded range in South Africa.

4.2.4 Species occurrence locations: refinement

Maps of occurrence locations for both native and invaded range data of *S. terebinthifolia* were produced in ARC Map 10.3 to check for obvious errors. Even though the data was known to be of high quality some “cleaning” was still conducted: (1) duplicates were removed, (2) where possible co-ordinate errors were corrected, and (3) co-ordinates lacking sufficiently fine-scale precision were excluded. Autocorrelation is an important factor that may affect model outputs. Filtering of species occurrence data may limit the inherent biases in the data and improve model quality (Veloz 2009). To avoid pseudo-replication only one occurrence record per 5 min grid cell was used for model calibration. Additionally the influence of spatial autocorrelation (spatial filtering) was checked using the ‘spThin’package (Aiello-Lammens et al. 2015). No spatial autocorrelation was detected therefore no further thinning and spatial autocorrelation analyses were performed.

4.2.5 Environmental parameters

Bioclimatic variables have been widely used in other niche-based plant distribution modelling studies. These can be freely downloaded from the WORLDCLIM database (www.worldclim.org) (Hijmans et al. 2005; Mukherjee et al. 2012), and several authors have recommended that the selection of variables be based on ecological knowledge of the taxon being modelled (e.g. Broennimann et al. 2007; Elith & Leathwick 2009; Elith et al. 2010; Austin & Van Niel 2011). For this study, climate data were obtained by downloading the standard set of bioclimatic variables from the WorldClim ver 2 database (Hijmans et al. 2005; 2016) (data available at: http://worldclim.org/version2). As *S. terebinthifolia* distribution has been previously modelled and its climatic drivers based on the plant’s biology are well understood (Papes & Peterson 2003; Wang & Wang 2006; Zhu et al. 2007), we selected variables that are thought to be of biological relevance to its distribution (Kumar & Stohlgren 2009; Mukherjee et al. 2012). These variables from the Worldclim database included: Bio 1-Annual mean temperature; Bio 4-Temperature seasonality; Bio 6-Minimum temperature of coldest month; Bio 7-Temperature annual range; Bio 12-Annual precipitation; and Bio 15-Precipitation seasonality. As the layers were previously determined to be representative for the species, it was not necessary to check if the layers were collinear. In order to make the environmental layer suitable for MaxEnt they were converted from Raster file format into an ASCII file in ArcMap 10.3 (ESRI 2018). Additionally, they were clipped to the suitable geographically defined background using the ‘Extract by Mask’ function in ArcMap 10.3 (ESRI 2018).This was done for each of the models

4.2.6 Geographically defined background

MaxEnt creates pseudo-absence data drawn randomly from a geographically defined background, from pixels (grid cells) lacking presence records. It is well understood that the size of the “background” from which pseudo-absence data are obtained can significantly influence model results (Anderson & Raza 2010; Thompson et al. 2011), and therefore it is recommended that the background should be restricted or at least representative of where the species could potentially occur. The background should ideally represent the geographic areas available to the focal species, omitting areas where species absence is due to historical factors, dispersal constraints and/or biotic interactions (Sanín & Anderson 2018). Therefore for the native range data it was not possible to determine the region that has been historically explored for *S. terebinthifolia,* so we restricted the background data to a convex hull drawn around the area known to have been extensively surveyed (Mukherjee et al. 2012). For the USA, the background was clipped to match the spatial extent of Florida. In Florida *S. terebinthifolia* has been extensively mapped and studied. For South Africa, we used the entire country spatial extent, as we know the entire country has been repeatedly surveyed giving a good indication of the distribution of the species. To determine if when using the South Africa data we were overfitting the model we modelled the potential distribution of *S. terebinthifolia* using areas that were climatically representative i.e. the Koppen- Geiger climate classifications (Koppen-Geiger classifications, following the rules defined in (Kriticos et al. 2011) as recommended by Hill and Terblanch (2014). The climate data was downloaded from (http://koeppen-geiger.vu-wien.ac.at). The data were placed into ARCMAP 10.3 and the occurrence localities of *S. terebinthifolia* were layered over the data layer to find what climate zones contained at least one occurrence record and these climate zones were selected out to use as background data to the MaxEnt model. The Koppen- Geiger background was also clipped to areas within South Africa.

4.3 Modelling parameters in MAXENT

MaxEnt creates pseudo-absence data drawn randomly from a geographically defined background, from pixels (grid cells) lacking presence records. It is well understood that the size of the "background" from which pseudo-absence data are obtained can significantly influence model results (Anderson & Raza 2010; Thompson et al. 2011), and therefore it is recommended that the background should be restricted or at least representative of where the species could potentially occur. The background should ideally represent the geographic areas available to the focal species, omitting areas where species absence is due to historical factors, dispersal constraints and/or biotic interactions (Sanín & Anderson 2018). Therefore for the native range data it was not possible to determine the region that has been historically explored for *S. terebinthifolia*, so we restricted the background data to a convex hull drawn around the area known to have been extensively surveyed (Mukherjee et al. 2012). For the USA, the background was clipped to match the spatial extent of Florida. In Florida *S. terebinthifolia* has been extensively mapped and studied. For South Africa, we used the entire country spatial extent, as we know the entire country has been repeatedly surveyed giving a good indication of the distribution of the species. To determine if when using the South Africa data we were overfitting the model we modelled the potential distribution of *S. terebinthifolia* using areas that were climatically representative i.e. the Koppen- Geiger climate classifications (Koppen-Geiger classifications, following the rules defined in (Kriticos et al. 2011) as recommended by Hill and Terblanch (2014). The climate data was downloaded from (http://koeppen-geiger.vu-wien.ac.at). The data were placed into ARCMAP 10.3 and the occurrence localities of *S. terebinthifolia* were layered over the data layer to find what climate zones contained at least one occurrence record and these climate zones were selected out to use as background data to the MaxEnt model. The Koppen- Geiger background was also clipped to areas within South Africa

4.4 Modelling parameters in MaxEnt

MaxEnt allows the user to change a number of the modelling parameters to create the most suitable model possible, but it is not necessary to change the majority of the parameters as MaxEnt can automatically calculate the best parameters to be used and often the default parameters are suitable. For this study we used 'Auto features' or automatic constraints which automatically calculates how complex (either a linear or quadratic analysis) the model can be based on the number of occurrence locations. Additionally the MaxEnt parameters of 500 iterations and 0.00001 convergence threshold were used when running the program ‘logistic output’, which creates a continuous, linear scaled map that allows fine distinctions to be made between the modelled probabilities of habitat suitability. Also used in the models were ‘Create response curves’ and ‘jackknife measures of variable importance’. Additionally, ‘do clamping’ which resets values that are outside the range found in the study area to match the upper or lower values found in the study area, and a regularization value of 1, were applied, to avoid overfitting (restricting the number of locations to 1 per grid cell). There can be considerable variation in the performance of models when choosing a particular random selection of points for the training and testing sets, so a bootstrap selection was made which is good for relatively large datasets as it is best to make several random selections (splits). This was done five times for each model so that the models could be compared for consistency. The independent ‘testing data set’, used to test the accuracy of the model, comprised 30% of the occurrence localities and was found to be the best split (Trethowan et al. 2011). The split of 70% training and 30% testing was done randomly.The final model produced was the mean of the five models created. The mean minimum training presents logistic value was used from the five maps produced.

When only the native range data was used to predict the potential distribution in South Africa and when only the South African data was used to predict the native range, the “projections layers” feature was used. This projects the data that was used in the training of the model into a new geographic range that was not used in the training of the model to avoid the problem of confounding the model with too large a geographic background.

4.5 Model evaluation

Model evaluation is an essential component of the model building and interpretation process, but it is often neglected. Model assessment allows the user to objectively assess the quality of the model’s predictions. Without an objective assessment, the accuracy of the model is unknown. MaxEnt allows for a number of methods to evaluate the accuracy of the model. In this study, the models were evaluated using Jackknife analyses and response curves, with the Area Under Curve (AUC) statistic for each replicate within each treatment. The AUC has been found to be robust for uneven prevalence in observations of occurrence that can produce artefacts in other performance measures (McPherson et al. 2004). Consequently, in evaluating presence-only models, the ability to give a realistic prediction of species occupancy potential in the study area is considered to be more important than model accuracy (Lobo et al. 2008). Detailed descriptions of these AUC curves and Jackknife analyses appear in Pearson et al. (2007). The AUC values generated from the model can range between 0.5 and 1.0, where 0.5 is no better than a randomly generated model. It is generally accepted that an AUC of less than 0.8 is a poor model, between 0.8 and 0.9 is a fair model, between 0.9 and 0.995 a good model, and >0.995 an excellent model (Fielding & Bell 1997; Trethowan et al. 2011). The mean and standard deviation of the five replicate AUC values was provided for each model and used to determine the quality of the models. As the AUC has received some criticism (Pearson et al. 2007; Lobo et al. 2008; Merow et al. 2013; Radosavljevic & Anderson 2014), the extent of overfitting for each set of models produced as a measure of model accuracy was also investigated. This was done by comparing the omission rates with theoretical expectations of omission rates. The omission rate is the proportion of test occurrence localities that are not predicted to fall within the projected model surface once the model is converted into a binary prediction output (Boria et al. 2014). Over fit models, have omission rates higher than the theoretical expectation for the threshold applied (Shcheglovitova & Anderson 2013). The 10th percentile calibration omission rate was applied in this study to estimate model overfitting. This sets the binary prediction threshold at a value that excludes the 10% of the calibration localities from the model with the lowest prediction values, and therefore has an expected omission rate of 0.10 (Boria et al. 2014). Above 0.1 indicates an over fit model and below 0.1 being more conservative (Boria et al. 2017).

In the model calibrated using the invasive range South African, Florida and native range haplotype A occurrence locations, with a different set of environmental parameters, the Jacknife method of evaluation was used on the 19 WORLDCLIM bioclimatic variables to determine which layers were contributing the most to the model. Jackknife analysis is effectively the “leave one out” analysis. The model is re-run leaving out a variable allowing the programme to quantify the contribution of each variable. The model was then re-run with the five environmental layers that contributed the most to the model; these were then compared to the suggested climatic variables used by Mukherjee et al. (2012).

Although MaxEnt results can be seen in a picture format, greater visualization and detail is possible in ArcMap 10.3 (ESRI 2018). The MaxEnt images were opened in ARCMAP and converted into Maps. The ASCII raster layer was changed into classified values, where the minimum training presence logistic threshold was set as the upper limit of one of two categories. This gives the potential distribution map (effectively a presence-absence map).

4.6 Results

In South Africa *S. terebinthifolia* has primarily been recorded in the Indian Ocean coastal belt and savannah biome (Macina et al. 2006). At a provincial level, the majority of these localities fall in KwaZulu-Natal Province, with occasional records in the Eastern and Western Cape provinces. Figure 4.1 (a) shows the known distribution of *S. terebinthifolia* in South Africa. When modelling with the distribution records, to avoid pseudo replication, only one location point per grid cell was used (Joyner et al. 2010), effectively reducing the number of locality points used to calibrate the model. The revised number of localities used to calibrate the models are presented in Table 4.1.

4.6.1 South Africa distribution data

This model was calibrated using the six environmental parameters suggested by Mukherjee et al. (2012) and the 76 occurrence localities from South Africa (Figure 4.1a). This model indicated the current distribution closely matched the modelled areas of highest suitability (Figure 4.1.b, c). KwaZulu-Natal is indicated as the province with the highest suitability, but the coastal belt of northern Eastern Cape Province is also suitable for *S. terebinthifolia*. As the training data (occurrence localities) were regarded as good quality, all the training localities could be used to make an accurate prediction. The logistic value was 0.005 (which is the predicted area suitable for *S. terebinthifolia*) translating only 24 % (SE± 0.02) of the viable extent (Figure 4.1d). Using the minimum training presence, a larger area of South Africa is predicted than where the plant is currently recorded, and includes further coastal regions of the Eastern Cape Province. The mean AUC statistic for the model was 0.975 ± SE 0.002 indicating it was a “good” model (Figure 4.7). The Omission rate was 0.86 ± SE 0.02 indicated the model was not over fit (Not overestimating the potential area suitable)

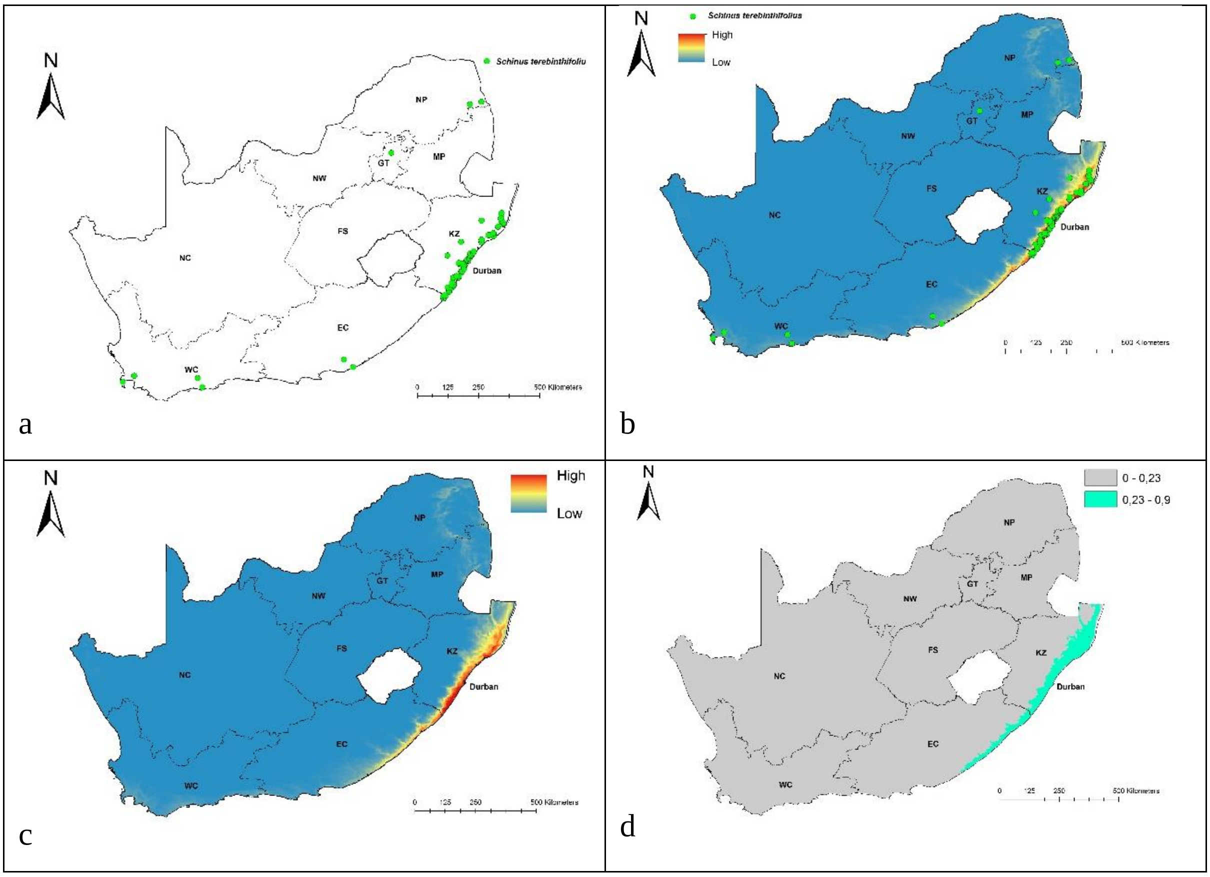


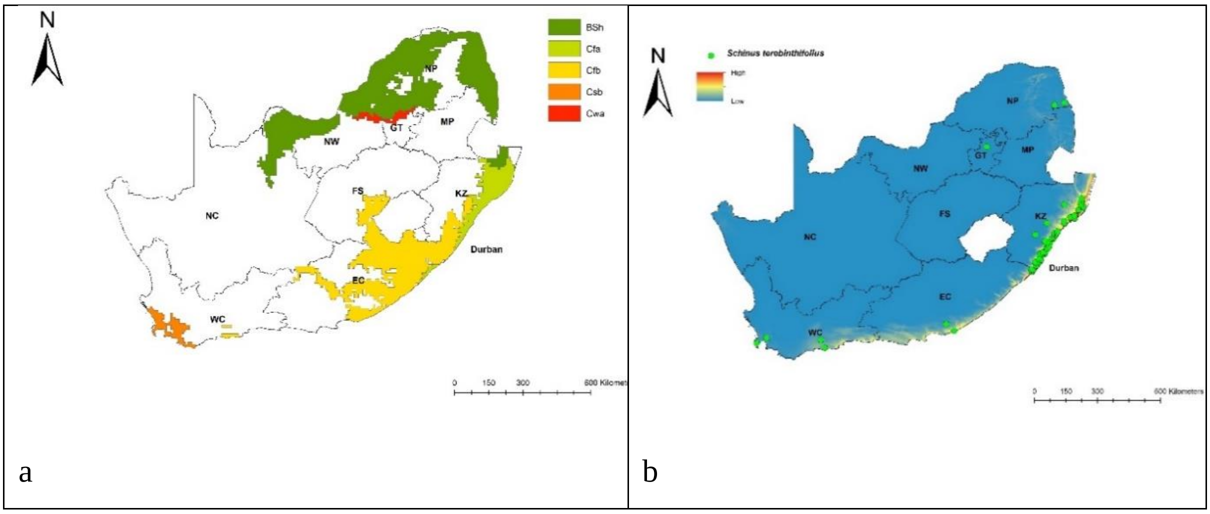
Figure 4.1 The potential distribution of *Schinus terebinthifolia* in South Africa using only the South African invasive range distribution points to calibrate the model.(a) Distribution sites from South Africa (b) predicted distribution with known localities overlaid (c) predicted distribution in South Africa (d) presence-absence distribution map of South Africa.

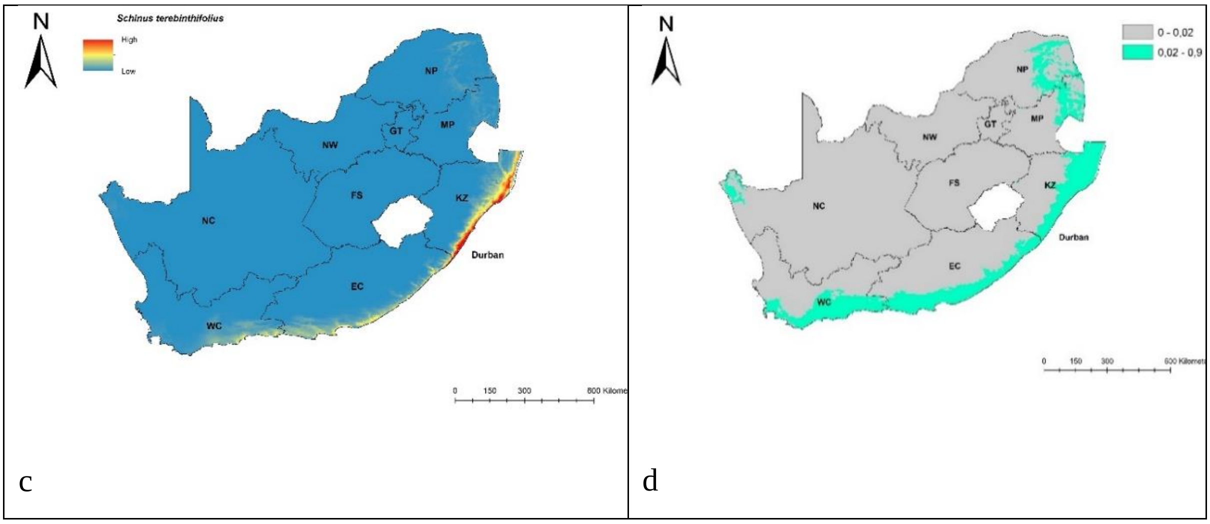
Table 4.1 Treatments and the refined number of training and testing data used to calibrate the final *S. terebinthifolia* models. Treatment: SAm: South America; SA: South Africa; SAKG- South Africa Koppen Geiger, SA,FL- South Africa and Florida; SA,Fl,SAm- South Africa, Florida, South America: SA,Fl,SAm1 -South Africa, Florida, South America with different climatic variables.

Treatment	Training	Testing	Total
SAm	60	25	85
SA & SAKG	54	22	76
SA,FL	182	77	259
SA, FL, SAm	244	104	348

SA, FL, SAm1	244	104	348

Using the whole of South Africa as a background may have created over fit models therefore the background was limited to areas that were climatically representative. These models were run once again using just the Koppen Geiger climate Zones from South Africa where *S. terebinthifolia* were located. These were: semi-arid climates (BSh), warm temperate with hot summer climate zones (Cfa), marine west coast (Cfb), warm-summer mediterranean climate (Csb), and warm temperate with hot summer climate (Cwa). The models once again predicted a limited area to the eastern part of South Africa (Figure 4.2b, c & d). The model calibrated using the minimum training presence 0.023 SE ± 0.04 shows a larger suitable area along the coast than using the of whole South Africa as a background. The AUC for the models were 0.958 SE ± 0.005 indicating it was a “good” model (Figure 4.7). The Omission rate was 0.86 ± SE 0.02 which indicated the model was not over fit, however, they were not considerably different from the backgrounds using the whole of South Africa.





c

d

Figure 4.2 The potential distribution of *Schinus terebinthifolia* in South Africa using the South African invasive range distribution points as well as Koppen- Geiger climate regions representative in South Africa to calibrate the model. (a) Clipped Koppen-Geiger climate regions for South Africa (b) predicted distribution with known localities overlaid (c) predicted distribution in South Africa (d) presence-absence distribution map of South Africa.

4.6.2 South Africa and Florida distribution data

The third treatment used two sets of species occurrence inputs from the weed's invaded ranges; (1) 76 species occurrences across South Africa, and (2) 183 species occurrence data points from Florida, USA (Table 4.1; Figure 4.3a). The model predicted a similar distribution to the invaded range South African data. The minimum training presence logistic threshold of 0.042 gives an average of climatically suitable area at a reduced 32 % (SD± 0.04) of the total available, more than the South African data only (Figure 4.3d). The additional localities where the species was recorded as growing in the USA increased the potential distribution in South Africa to include more inland regions of KwaZulu-Natal and a larger extent of the Eastern Cape coastline, as well as some areas in Limpopo Province (Figure 4.2b & 4.2c). Small areas in the Western Cape were also shown to be suitable. The AUC for the model was 0.963 SE ± 0.003, indicating a "good" model (Figure 4.4). The omission rate was 0.8 ± SE 0.02 which indicated the model was not over fit.

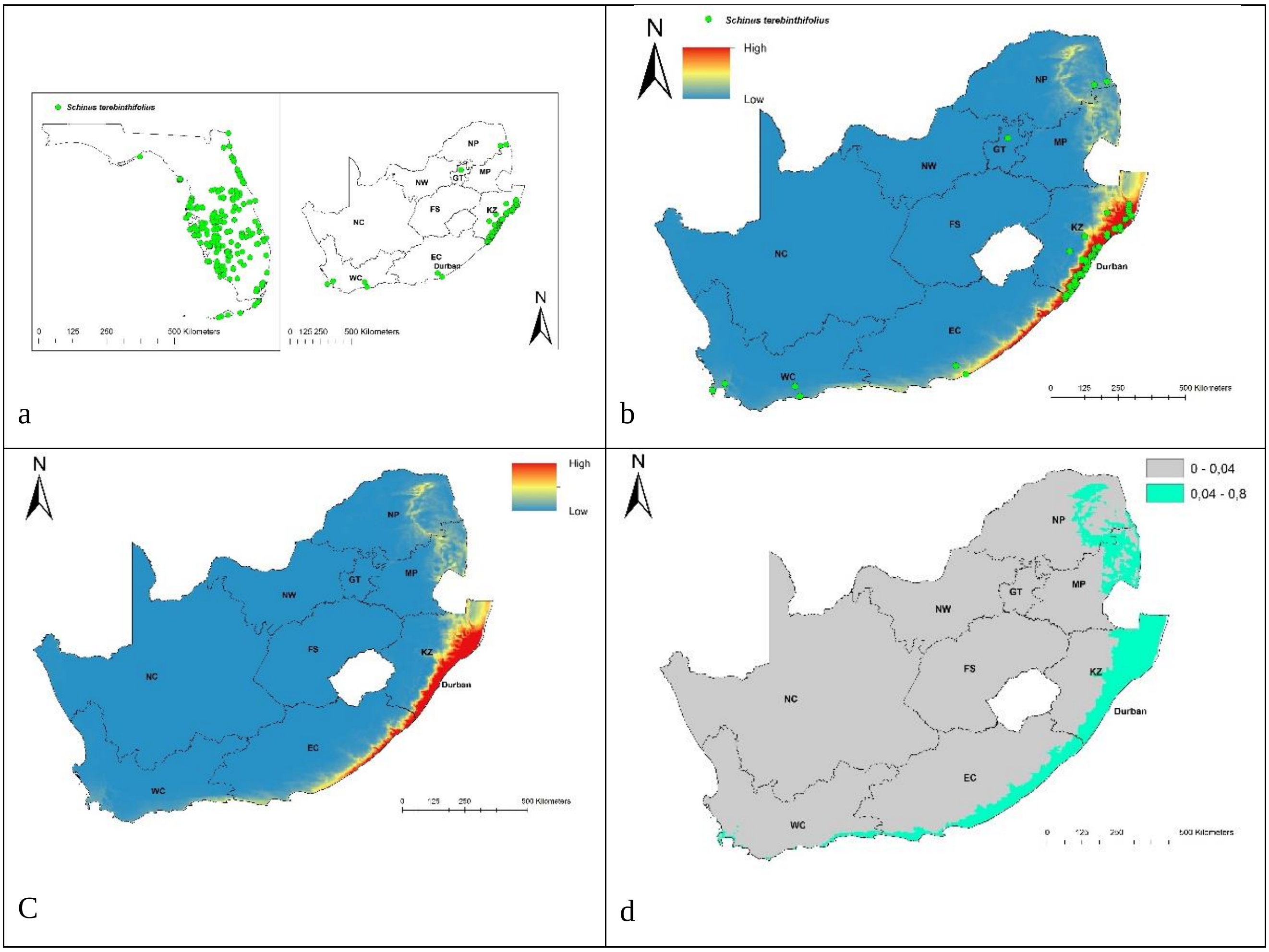


Figure 4.3 The potential distribution of *Schinus terebinthifolia* in South Africa using the South African and Florida, USA, invasive range distribution points to calibrate the model. (a) Distribution of sites from South Africa and Florida (b) pedicted distribution in South Africa with known localities overlaid (c) predicted distribution in South Africa (d) presence-absence distribution map of South Africa

4.6.3 South Africa, Florida and native range data

The fourth treatment used 76 species occurrence data sets from South Africa, 183 species occurrence data points from Florida, USA, and 89 haplotype A occurrence localities from specific surveys conducted in the native range of Brazil, Uruguay and Argentina (Figure 4.4a). The model had a minimum training presence of 0.005 giving average area climatically suitable for *S. terebinthifolia* at 56% (SD± 0.02) based on the model inputs. This increased the suitable area in South Africa to include areas more inland, including Limpopo and Mpumalanga provinces (Figure 4: b, c &d), and increased areas of the Western Cape. The model had the AUC 0.949 ± SE 0.002 (Figure 4.4). The omission rate was 0.11 ± SE 0.01 that indicated the model was slightly over fit.

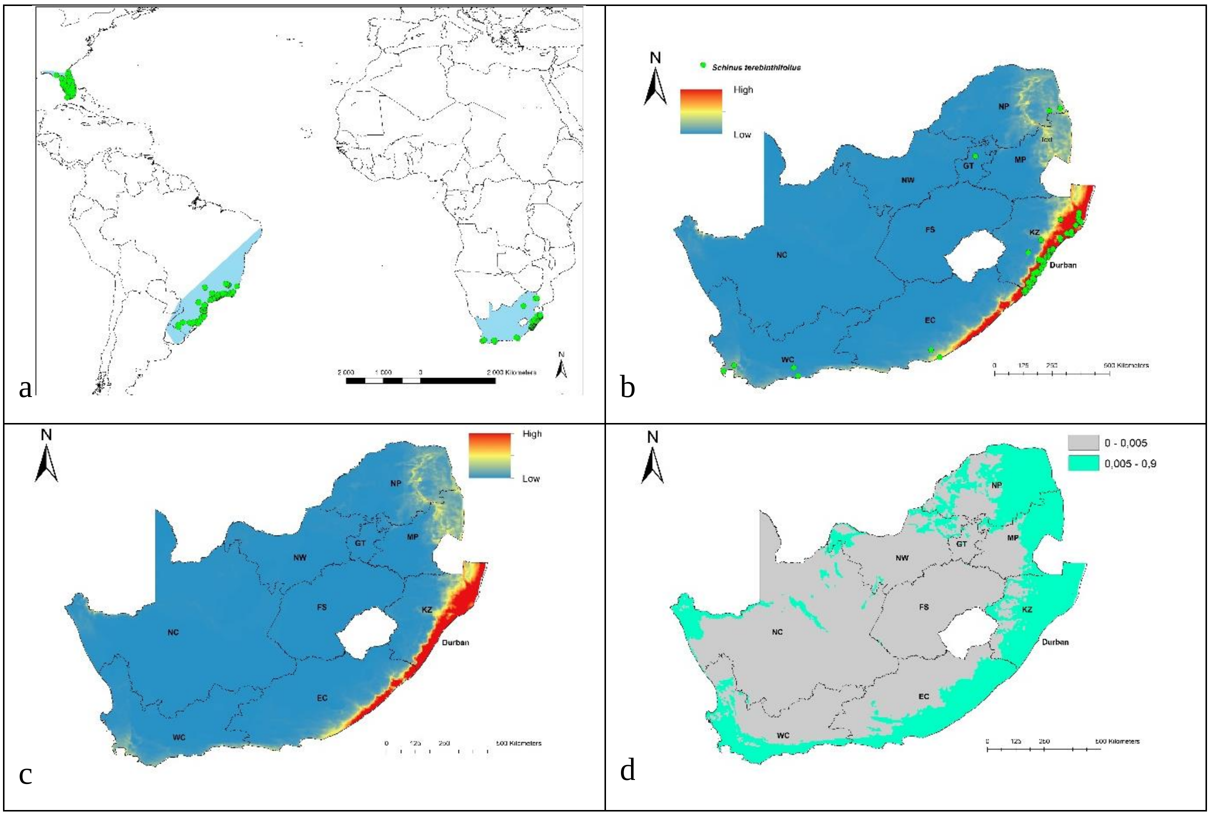


Figure 4.4 The potential distribution of *Schinus terebinthifolia* in South Africa using native range and invaded range distribution points to calibrate the model. (a) Distribution of sites from South Africa, Florida and South America (b) Predicted distribution in South Africa with known localities overlaid (c) predicted distribution in South Africa (d) presence-absence distribution map of South Africa.

4.6.4 South Africa, Florida and native range data with different climatic variables.

The fifth model once again used all the locality data, but used different environmental layers than the layers recommended by Mukherjee et al. (2012) (Figure 4.5a). The Jackknife analysis on the environmental layers suggested that variables contributing the most to the distribution of the species were BIO8 - Mean temperature of wettest quarter; BIO17 - Precipitation of driest quarter; BIO15 - Precipitation seasonality (coefficient of variation); BIO6 - Min temperature of coldest month; BIO4 - Temperature seasonality (standard deviation *100); and BIO3 - Isothermality (BIO2/BIO7) (* 100) (Table 4.2). Three layers were similar to Mukherjee et al. (2012), but :BIO8 - Mean temperature of wettest quarter; BIO17 = Precipitation of driest quarter and BIO3 - Isothermality were suggested as contributing more to the model than: BIO 1 - Annual mean temperature, BIO 7 -Temperature annual range and BIO 12 - Annual precipitation. The AUC for the model was 0,957 SE±0,003 once again indicating a “good” model. The omission rate was 0.11 ± SE 0.01 indicated the model once again was slightly over fit.

Table 4.2 Bioclimatic variables used in model calibration as well as the percentage contribution of each variable to the model. The biological variables suggested by Mukherjee et al. (2012). Shared bioclimatic variables are highlighted in blue.

Variable	**Percentage contribution**	**Permutation importance**	**Variable suggested by** (Mukherjee et al. 2012)
BIO8 = Mean temperature of wettest quarter	32.6	6.2	BIO 1 = Annual mean temperature
BIO17 = Precipitation of driest quarter	20.3	8.8	BIO 12 = Annual precipitation
BIO15 = Precipitation seasonality (coefficient of variation)	10.1	3.5	BIO = 15 Precipitation seasonality
BIO6 = Min temperature of coldest month	6.6	6.3	BIO 6 = Min. temperature of coldest month
BIO4 = Temperature seasonality (standard deviation *100)	6.3	7.4	BIO = 4 Temperature seasonality
BIO3 = Isothermality (BIO2/BIO7) (* 100)	5.1	24	Bio 7-Temperature annual range

The models once again indicated a suitable climatic areas biased to the eastern coastline of South Africa. However, compared to previous models this prediction favoured a more northerly distribution in South Africa with greater amounts of suitable area available in Northern KwaZulu-Natal and Limpopo Province and less in the Eastern and Western Cape provinces (Figure 4.5 ab& c). Of note was a small region along the Orange river in the Northern Cape Province that was also suitable (Figure 4.5d).

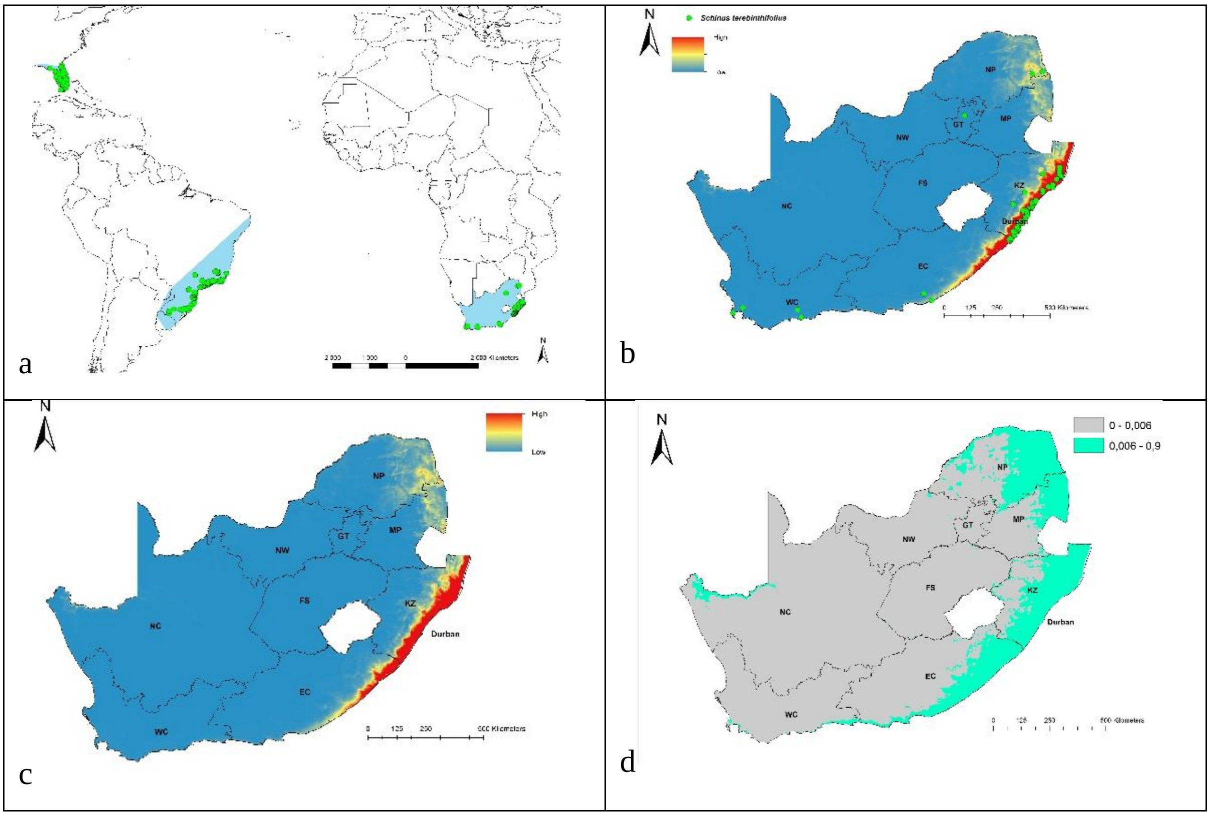


Figure 4.5 The potential distribution of *Schinus terebinthifolia* in South Africa using native range and invaded range distribution points to calibrate the model and alternative climatic variables. (a) Distribution of sites from South Africa, Florida and South America (b) Predicted distribution in South Africa with known localities overlaid (c) predicted distribution in South Africa (d) presence-absence distribution map of South Africa.

4.6.5 Native range data

The models, calibrated with native range data using only Haplotype A data and only clipped to the areas surveyed for *S. terebinthifolia*, showed the most restricted distribution in South Africa (Figure 6a). They indicated that only limited areas were suitable along the KwaZulu-Natal, Eastern Cape and Western Cape Province coastlines (Figure 4.6: b, c & d).

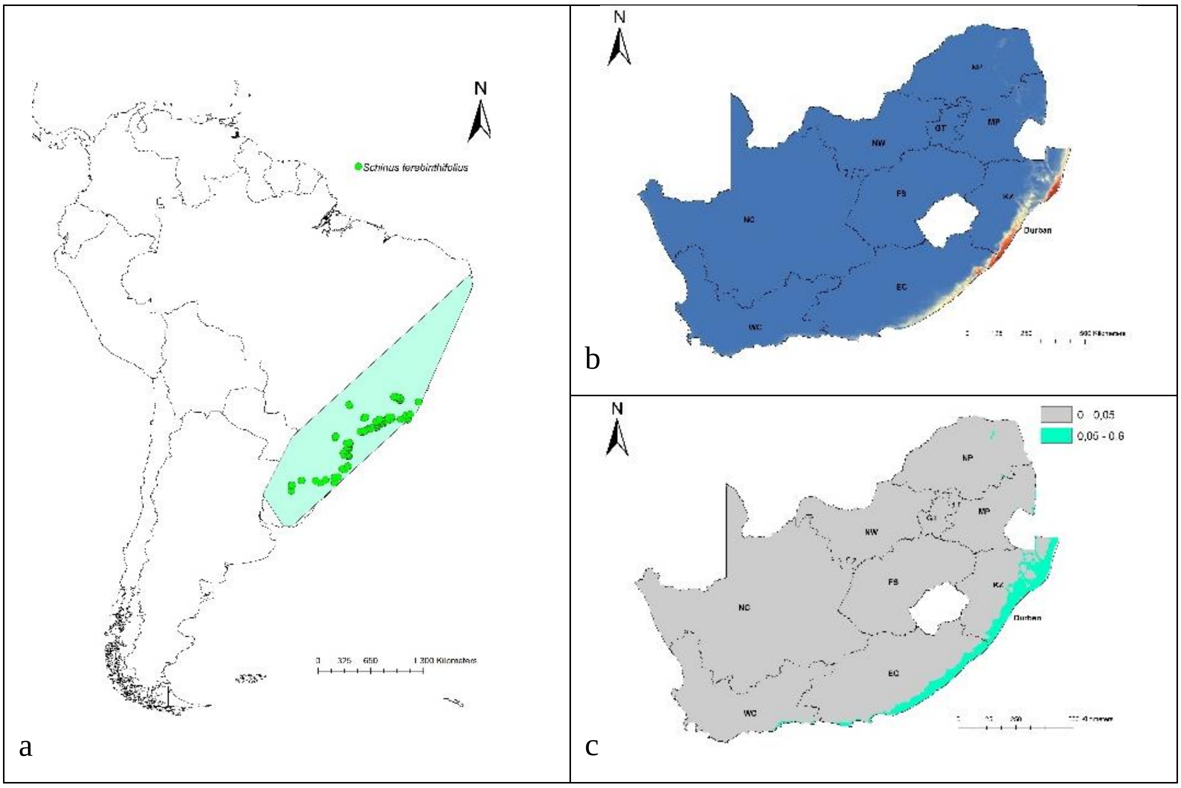
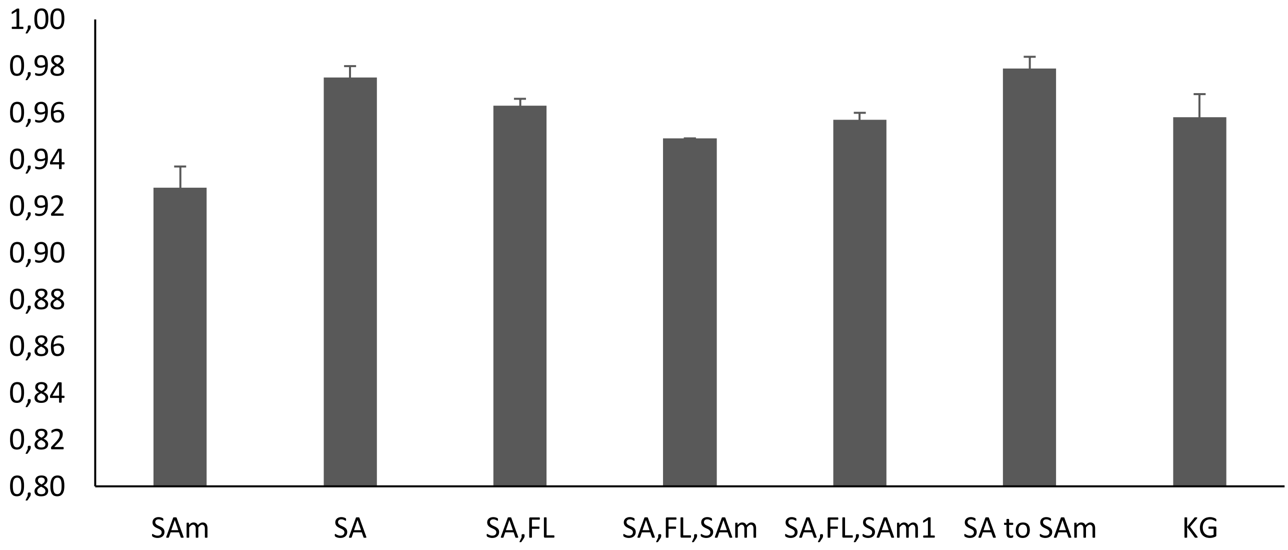


Figure 4.6 The potential distribution of *Schinus terebinthifolia* in South Africa using only the native range occurrence localities to calibrate the model. (a) Distribution of sites from South America (b) predicted distribution in South Africa (c) presence-absence distribution map of South Africa.



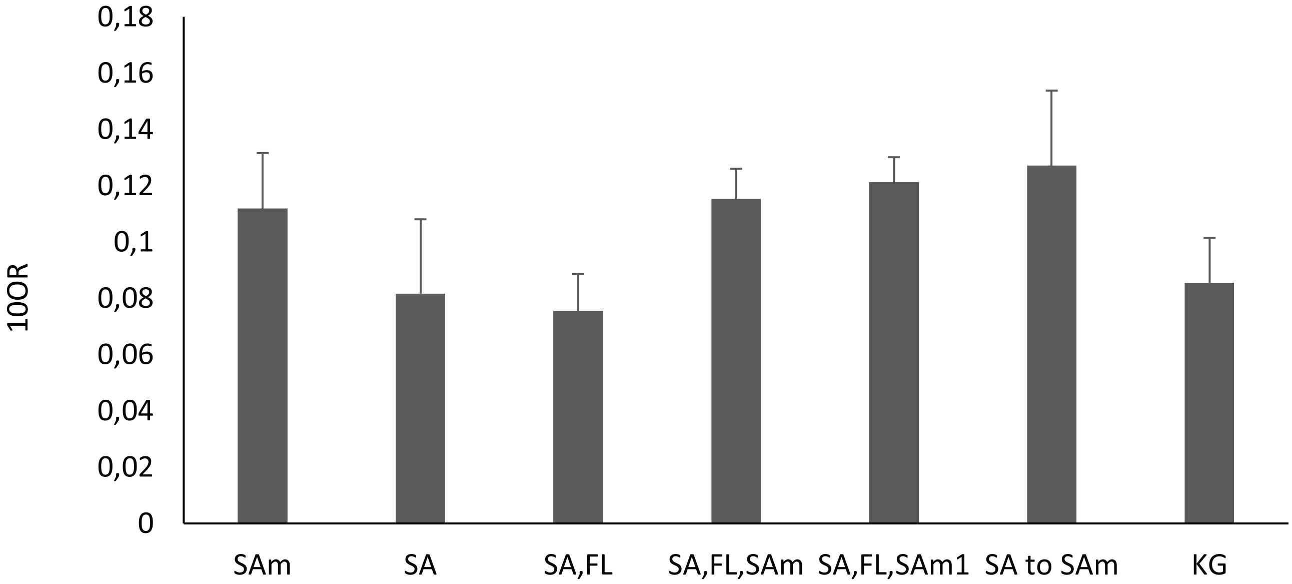


Figure 4.7a&b (a) Mean Area under Curves (AUC) with standard errors for *Schinus terebinthifolia* modelled treatments. Treatments: SAm- South America; SA- South Africa; SA,FL- South Africa and Florida; SA,Fl,SAm- South Africa, Florida, South America; SA,Fl,SAm1 -South Africa, Florida, South America with different climatic variables. Values of less than 0.8 indicate a poor model, between 0.8 and 0.9 is a fair model, between 0.9 and 0.995 a good model, and >0.995 an excellent model (Fielding & Bell, 1997; Trethowan et al. 2011). Figure 4.7b. The 10th percentile calibration omission rate (OR10) used to estimate model overfitting.

OR10 sets the binary prediction threshold at a value that excludes the 10% of the calibration localities from the model with the lowest prediction values, and therefore has an expected omission rate of 0.10 (Sutton 2019).

4.6.6 Prioritised regions to survey for potential agents in the native range

Matching areas in the native range to similar climates experienced in the invaded range means selected agents are more likely to establish and proliferate if released into the invaded range. Areas in the native range identified as being climatically similar to the South African *S. terebinthifolia* localities include coastal regions of Brazil, and specifically regions between the cities of Florianopolis and Santos (Figure 4.6). Additionally, those regions where haplotype A, the same haplotype as recorded in South Africa, was sampled are depicted by green circles in Figure 4.8. The AUC for the model was 0.979 SE ±0.005, which indicated a good model. The omission rate was 0.12 SE± 0.02 indicating a slightly over fit model (Figure 4.7). This is not concerning as highlighting larger areas for surveying would allow for a greater area to be assessed for potential biological control agents. This model should be overlaid with haplotype A distributions from the native range.

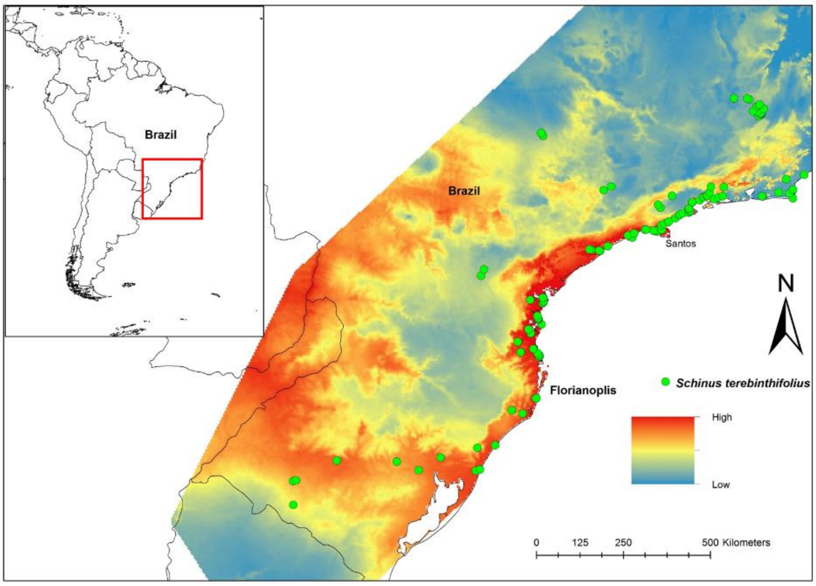


Figure 4.8 Areas of similarity in the native range of *Schinus terebinthifolia* in South America to its current South African distribution, calibrated using South African invaded range data.

4.7 Discussion

Species distribution models are a very popular tool in ecology and biogeography and have great potential to help direct conservation efforts (Newbold et al. 2010). These models produce climate suitability maps from available distribution data using a set of model parameters determined by the researcher. Accuracy and relevance of these data can be biased and leave room for interpretation. Essentially, no models are perfect, but they can be extremely valuable in both understanding the drivers of species establishment and persistence, and in assisting with invasive species management (Trethowan et al. 2011).

This study highlighted areas suitable in South Africa for *S. terebinthifolia* based on the known native and invaded distributions of the species. Due to previous research conducted by Mukherjee et al. (2012), as well as the genetic studies conducted in Chapter 3, this study was able to refine the predictive models to a specific haplotype of the species, thereby increasing model accuracy. The majority of the models predicted areas suitable for *S. terebinthifolia* in South Africa and highlighted the areas where the species has already established and is regarded as a troublesome invader. These areas include the coastal regions of KwaZulu-Natal Province and northern coastal areas of Eastern Cape Province. The addition of information from where the tree is already invasive from Florida, USA, and data from the tree's native range in South America, increased the areas predicted as suitable in South Africa. This increased area included areas further inland in KwaZulu-Natal and Eastern Cape provinces but also added areas of the Western Cape and Limpopo provinces. Most of the models indicated large areas in South Africa are suitable for *S. terebinthifolia,* suggesting that the tree has not yet reached its full distribution and will continue to spread in South Africa. However, as with all models, the quality of the input data and the parameters used within the model should always be carefully considered.

Understanding the limitations and strengths of modelling programs can help in interpreting the final outputs (Merow et al. 2013). MaxEnt is regarded as one of the premier distribution modelling software packages available, as it performs well in comparison with other correlative approaches (Elith et al. 2011; Thompson et al. 2011; Sutton 2019). Additionally MaxEnt has been used in a number of invasive species models with great success (Phillips et al. 2017). The use of these modelling programmes is assisted by the wealth of species distribution data that is now easily available (Beck et al. 2014). However, all distribution data should be treated with circumspection. Error can be introduced at any stage. Some basic types of errors include species misidentification, error within cataloged herbarium records and data capturing errors all of which result in inaccurate distributions and can significantly influence model accuracy. The surveys in its native range (Williams et al. 2005; 2007; Mukherjee et al. 2012), as well as the rigorous sampling conducted in the invaded range of Florida, USA, ensured that the input localities were comprehensive and accurate. There was also no sampling bias and error in the distribution data. Additionally, models calibrated using data from both the native range of the species and the invaded range provide a better indication of a species' potential to spread (Trethowan et al. 2011). As with distribution data, environmental layers used by MaxEnt are also often freely available for example WorldClim ver 2 database (Hijmans et al. 2005; 2016) (data available at: http://worldclim.org/version2). These data also need to be treated carefully. This was taken into consideration and the background of every population was refined. Additionally, two different types of layers were attempted: one using biologically representative layers for *S. terebinthifolia* layers as used by Mukherjee et al. (2012) and a second based on the best six layers after conducting a bootstrap analysis on the 12 availible environmental layers. Interestingly, these were similar to the layers used by Mukherjee et al. (2012).

Different environmental layers changed the predicted potential distribution in the northern parts of KwaZulu-Natal and Limpopo provinces, but the east coast of South Africa remained as the most suitable. This was driven by precipitation in the wettest and driest seasons, which was not, included in Mukherjee et al. (2012) environmental layers. Once the distribution data and environmental layers are developed, correlative models can be relatively easily produced and are repeated until relevant models are produced (Beck et al. 2014). Once produced, their outputs can be further integrated through a number of methods. According to McPherson et al. (2004), AUC has been found to be the most robust method for predicting model success. The MaxEnt predictions of *S. terebinthifolia* distribution were highly accurate (ranging between 0.93 and 0.98) suggesting good and excellent models (Beaumont et al. 2009; Trethowan et al. 2011). Also in this study, the 10th percentile calibration omission rate was also investigated to determine model accuracy. Models with omission rates increasingly higher than the expected value were considered as more over fit (Boria et al. 2017). However, the majority of the OR 10 values were near to one, indicating the models were very close to accurate, but with four models slightly over fit and three models under fit. Once again, the predicted distributions of the over fit and under fit models suggested similar areas in South Africa that are suitable to invasion.

Of interest was the increase size of suitable regions on the maps as additional data points were included into the models. The models produced using just the South African localities predicted a suitable band down the east coast of South Africa, primarily in KwaZulu-Natal Province. The addition of invaded range data from Florida, USA increased the predicted potential distribution in South Africa to a greater percentage of the east coast of South Africa, moving down into the Eastern Cape Province and just slightly into the Western Cape Province. The addition of the native range data to the invaded range data increased the potential distribution in South Africa to the entire eastern coastline of South Africa, including the Western Cape Province. This model is probably the most accurate potential distribution map of *S. terebinthifolia* in South Africa as it uses the most relevant environmental layers as well as all the available distribution data for the species. This is expected as considering both native and invaded range localities has been shown to increase model accuracy (Robertson et al. 2008; Trethowan et al. 2011; Scott et al. 2016). Refining the available distribution data to include only the relevant haplotype data is also likely to have improved the accuracy of the models.

As stated in Chapter 1 *S. terebinthifolia* was a common ornamental species in South Africa, which was sold within the nursery trade before it escaped and became invasive. In some cases, when ornamental species escape cultivation there has been an increase in genetic diversity. This occurs when sexual reproduction takes place between individuals of the same species imported from different regions of its native range and/or from regions of the world where the species has also been sold (Kwong et al. 2017; Chapter 3). This increased genetic variability in the invaded range can then allow rapid adaptation into new niches (Williams et al. 2007; Mukherjee et al. 2012). When this happens, predicting their potential distribution becomes very difficult. This has occurred with *S. terebinthifolia* in Florida, USA, where two haplotypes of *S. terebinthifolia* hybridised and this allowed the species to increase its fundamental niche (Mukherjee et al. 2012). Mukherjee et al. (2012) suggested that one haplotype of *S. terebinthifolia* was initially able to establish in Florida due to similar climate compatibility between Florida and the tree’s native range in South America. However, they suggest the tree was then able to spread to areas regarded to be out of the individual haplotypes fundamental niche due to intraspecific hybridisation (Mukherjee et al. 2012). A common garden experiment conducted in Florida showed that that hybrid *S. terebinthifolia* had higher survival, growth rates and biomass than non-hybrids (Geiger et al. 2011). Mukherjee et al. (2012) continued to suggest that multiple introductions of various haplotypes might assist invasion success (Geiger et al. 2011; Mukherjee et al. 2012). However, Chapter 3 showed that only a single haplotype,

"haplotype A", is present in South Africa. This highlights the importance of ensuring no other haplotypes are introduced into South Africa as it may result in *S. terebinthifolia* hybridising and being able to expand its climatic niche.

Schinus terebinthifolia was originally only declared a category 1b weed in KwaZulu-Natal Province as it was suggested that this was the only region suitable for the establishment and spread of the species in South Africa. A category 1b invasive species by law requires a management plan for its control and should be actively managed. In the rest of the South African provinces, it was deemed a less serious invasive and declared a category 3 invader (Henderson 2001). This was updated in National Environmental Management: Biodiversity Act (NEMBA) (10/2004) in 2004 where it got elevated to a category 1b in the Eastern Cape, Limpopo, Mpumalanga and KwaZulu-Natal provinces. This change in legislation was supported by the predictive models which predicted areas out of the current distribution in KwaZulu-Natal and into the other provinces. It is of concern that *S. terebinthifolia* is not listed as a category 1b in the Western Cape Province. Every modeled treatment, with the exception of the first treatment which just used the South Africa distribution data, predicted at least part of the Western Cape Province to be suitable for invasion by *S. terebinthifolia*. The treatment using the Koppen Geiger climate regions for background data suggested large amounts of the Western Cape Province to be suitable for the invasion of *S. terebinthifolia*. If *S. terebinthifolia* reaches the Western Cape Province, especially the coastal region, it may have similar impacts as in KwaZulu-Natal, where managers are struggling to manage the spread of the species.

In KwaZulu-Natal Province, primarily in the eThekwini municipality, *S. terebinthifolia* has been extensively managed using both chemical and mechanical control measures, including using the registered chemicals Garlon or Timberel (www.botanicalsociety.org.za). These control measures have not been sufficient to control populations of the tree and it continues to spread both within populations and to develop new populations. Therefore, biological control should be considered as a sustainable viable long-term solution for the species. In this study, MaxEnt was used to refine regions from the species native range that would be climatically similar to South Africa populations of the tree. Predictive models have been used to assist in identifying areas climatically suited to priorities areas to conduct native range surveys for biological control programmes (Paterson et al. 2009; Trethowan et al. 2011). Sutton (2019) suggested a lack of climatic compatibility between the source of the invader and the invaded range as a limiting factor in the success of biological control agents. Natural enemies found in areas of the native distribution where climatic conditions are similar to those in the introduced distribution are more likely to establish and persist (Robertson et al. 2008). This study indicated that regions in the plant's native range between Florianopolis and Santos in south-eastern Brazil were climatically similar to areas where the tree has invaded in South Africa. These areas found in the native range also harbour haplotype A that is currently invading South Africa. This area is therefore the most appropriate region to survey for climatically and genetically matched potential biological control agents.

5 Chapter 5: General discussion

This study aimed to investigate select drivers behind the invasion of *S. terebinthifolia*, with the ultimate goal of providing solutions to facilitate in its management in South Africa. The tree has been in South Africa for over a decade and has since escaped cultivation and spread to become a NEMBA category 1b and 3 listed invasive demanding management (NEMBA Act 10.2004). What makes the invasion of *S. terebinthifolia* unique is that the species acquired a specialist native seed predator *M. transvaalensis* (Grissell & Hobbs 2000). The first aim of this thesis was to quantify the impact of *M. transvaalensis* on *S. terebinthifolia* to determine if it was influencing the species in South Africa. Quantifying the impact of the wasps on *S. terebinthifolia* was motivated by the influence of the unintentionally introduced wasp on *S. terebinthifolia* populations in both Florida and Hawaii, USA. In these two states of the USA, the wasp is considered a valuable biological control agent that can damage up to 80% of viable seeds (Wheeler et al. 2001; Hight et al. 2002). It was found that *M. transvaalensis* was widely distributed in South Africa; however, a lower level of damage was recorded compared to the USA (Chapter 2). The low damage levels suggest that the wasp's impact is not significant enough to reduce the populations of the species in South Africa.

The different variants of *S. terebinthifolia* in the native range and the recorded intraspecific hybridisation of haplotype A and B in Florida, led to the investigation into the invasion history of *S. terebinthifolia* in South Africa. This was done to see if comparison could be drawn to the USA, where a hybrid of the weed has been shown to be a superior invader when compared to its progenitor haplotypes (haplotypes A and B). Only haplotype A was recorded in South Africa and no indications of any hybridisation was evident (Chapter 3). The South Africa population was also found to have a low number of alleles per loci, supporting the theory that the South African population derived from a single introduction, similar to the stock population introduced into Florida and Hawaii, or possibly originating in either Florida or Hawaii. Determining the introductory pathway into South Africa was important for prioritising areas to survey for any possible biological control agents (Goolsby et al. 2006; Cuda et al. 2019). It also provided information towards understanding the relationship between the *M. transvaalensis* and *S. terebinthifolia*, as the impact of the wasp on the different haplotypes was shown to vary in the USA (Geiger et al. 2011). Finally, the fourth aim was to determine if the full potential distribution of *S. terebinthifolia* in South Africa had been reached and where to survey for climatically suited potential agents in the native distribution. The models showed potentially suitable areas that are currently uninvaded by *S. terebinthifolia*, suggesting that the species will probably continue to spread in the country if left unmanaged (Chapter 4). One of the most important findings from the predictive models was that the legislation regarding *S. terebinthifolia* in the Western Cape Province should be changed, as the climate is suitable to support *S. terebinthifolia*. The coastal region between Florianopolis and Santos in south-eastern Brazil was identified as the closest climatic match to the invaded distribution in South Africa and is therefore the most appropriate region to source biological control agents.

Biological control is regarded as one of the premiere control measures currently implemented against certain invasive plant species in South Africa, especially for trees (reviews, see Hoffmann & Moran 1991; Olckers & Hill 1999; Moran et al. 2011). As of 2016, 773 alien plant taxa have been recorded as established in South Africa (Henderson & Wilson 2017). Unfortunately, according to Zachariades et al. (2017), only about 59 of these alien plants have a working biological control programme implemented against them, leaving the vast majority without biological control. Selecting which species should be prioritised for management, especially for weed biological control remains a difficult and contentious process. Globally, a number of different methods for prioritisation have been suggested (Van Klinken et al. 2016). The most common approach is to invite and query experts in the field for their opinion, as well as to search available literature (Martin et al. 2012). These data can then be transformed into various models or prioritisation methods namely: conceptual, semi-quantitative or quantitative (Van Klinken et al. 2016). Essentially all methods should be guided by solid fundamental research and data. This thesis should greatly contribute to any discussions regarding the management of *S. terebinthifolia* in South Africa

Historically, in South Africa, weed biological control was primarily funded through the South African Department of Agriculture and therefore the selection of weed candidates was, for obvious reasons, focused on agricultural weeds with the emphasis on cactus invaders. In 1994, weed biological control was moved out of the department of agriculture into the funding model of the Working for Water programme (WfW) now one of the Natural Resource Management Programmes (NRMP) within the Department of Environmental Affairs (DEA). This has led to substantial increases in the funding available for biological control of environmental weeds over the past 20 years (Zachariades et al. 2017). In South Africa no official prioritisation method for invasive alien plants exists prioritisation is usually is driven by expert knowledge and researched within the field, therefore there is no set method to ensure *S. terebinthifolia* receives funding for biological control. *Schinus terebinthifolia* is currently not prioritised for biological control, but the limited impact of *M. transvaalensis* on *S. terebinthifolia* in South Africa, the significant impacts seen in the USA, and the broad and potentially expanding distribution, as well as the significant amount of work already conducted towards developing biological control agents for the USA, indicates that *S. terebinthifolia* is a good target for biological control in South Africa.

When starting a biological control programme, it is important to determine whether potential agents are not already present in the introduced distribution. For example, when the biological control programme against *Sesbania punicea* (Cav.) Benth. (Fabaceae) was initiated in South Africa in 1982, four weevil species, including *Trichapion lativentre* Beguin-Bellecocq (Curculionidaea), were introduced into quarantine facility to undergo host specificity testing (Hoffmann & Moran 1991). However, retrospective surveys two years later revealed *T. lativentre* was already broadly distributed in South Africa (Hoffmann & Moran 1991). *Megastigmus transvaalensis* attacking the seeds of *S. terebinthifolia* has been known in South Africa since the 1998's (Grissell & Hobbs 2000) but the impact remained undetermined. The seed-attacking wasp was shown to damage seeds throughout the invaded distribution in South Africa, but in most cases, the level of damage was insignificant and far lower than in the USA. The damage of *M. transvaalensis* on *S. terebinthifolia* seeds in South Africa is similar to that of the natural predation of *Megastigmus aculeatus* (Hymenoptera: Torymidae) on *Rosa multiflora* (Rosaceae) (Jesse et al. 2013), where the natural levels of *M. aculeatus* predation are not sufficient to limit the reproduction of *R. multiflora* (Jesse et al. 2013). Why the wasp is not affecting *S. terebinthifolia* to the same level as in Florida and Hawaii, USA remains to be determined. It may be due to the wasp having a preferred alternative hosts from the *Rhus* genus in South Africa. Quantifying seed predation on *Rhus* hosts and comparing them with the *S. terebinthifolia* seed predation could investigate the question of an alternate host. It is, however, important to remember that the wasp is responsible for a certain level of damage to *S. terebinthifolia* in South Africa and may contribute to the overall level of control once new agents are introduced. This would be similar to the control of *S. punicea* where the unintentionally introduced *T. lativentre* now plays an important role in providing full control of the weed in combination with two other agents (Hoffmann & Moran 1991; 1999). Therefore, for *S. terebinthifolia* to be satisfactory reduced there should be a combination of natural enemies. In South Africa *M. transvaalensis* is not damaging enough to reduce the population of *S. terebinthifolia* but it could still assist in the management of the tree, as seed predators are often recommended as a first line of attack in biological control programmes (Hoffman & Moran 1998).

There is growing pressure on biological control practitioners to prioritise which potential agents will most likely be effective, before they are introduced in quarantine facilities (Morin et al. 2006). The initial stages of biological control programmes, including keeping insects in quarantine, are the most costly (Zachariades et al. 2017). This has resulted in an increased effort in the pre-release stages of the biological control programme pipeline (Paterson et al. 2014; McClay & Balciunas 2005; Morin et al. 2006). Investigations prior to the initiation of a biological control programme are often neglected and many questions, such as climatic and genetic suitability of the target weed populations, are usually only conducted if problems are encountered. For example, *Listronotus sordidus* (Gyllenhal) (Curculionidae) was imported into quarantine in South Africa as a potential biological control agent for the invasive macrophyte *Sagittaria platyphylla* (Engelm.) J.G. Sm (Alismataceae) (Martin et al. 2018). In quarantine, the agent was shown to be both host specific and damaging. However, investigations of the populations of *S. platyphylla* in South Africa, when compared to the native range, showed the *S. platyphylla* to be growing in different habitats. More importantly, habitats in South Africa were unsuitable for the establishment of *L. sordidus* and the programme was ultimately terminated (Martin et al. 2018). Therefore, any investigation that can prioritise or eliminate species before introduction into quarantine are considered helpful.

Climatic matching and genotype matching are two methods of prioritisation that can be considered before agent selection (Goolsby et al. 2006; Robertson et al. 2008; Paterson et al. 2009). The basic principle implies that biological control agents collected in areas of the native distribution, where climatic conditions are similar to those in the introduced distribution, are more likely to establish and persist (Robertson et al. 2008). For example S*olanum mauritianum* Scop. (Solanaceae) has a broad distribution in South Africa favouring the higher rainfall regions, particularly the Western and Eastern Cape, KwaZulu-Natal, Gauteng, Mpumalanga and Limpopo provinces (Olckers 2003; 2011). In 2008 a biological control agent *Anthonomus santacruzi* Hustache (Coleoptera: Curculionidae) was released in an attempt to reduce *S. mauritianum*'s flowering capacity, excessive seed production and dispersal (Olckers 2008; Klein 2011). Unfortunately, despite significant release efforts, the agent only established along a climatically limited area of the southern coastline of KwaZulu-Natal Province (Olckers 2011; Cowie et al. 2016). Retrospective climate matching has showed that regions where the insect had established in South Africa matched with a greater than 60%

accuracy to areas where the insects were collected from its native range, while areas where the agent had not established had a less than 50% match to the native range (Cowie et al. 2016). The agent will not have a significant impact on the *S. mauritianum* population in the majority of invaded localities in South Africa because it is climatically limited and new climatically suited agents need to be considered (Cowie et al. 2016). Conducting this climatic matching for the biological control programme against *S. mauritianum* prior to the importation of *A. santacruzi* may have resulted in the importation of a better suited agent, or a better suited lineage of *A. santacruzi*.

Prioritising regions to search for potential biological control agents can significantly refine the amount of time spent searching for agents but also ensure that agents collected are suited to the invasive population. Identifying the haplotype in South Africa, as well as having the haplotypes of the populations in the USA and South America, contributed to the predictive modelling in Chapter 4. If the distributions of the hybrid populations were unknown, modelling would have predicted areas larger than the actual niche of haplotype A, effectively influencing the size of the potential distribution in South Africa, and the size of the prioritised regions in South America to search for biological control agents. Currently *S. terebinthifolia* distribution is limited to the tropical coastal regions of South Africa, however, it is predicted this may change to include the Mediterranean climates of the Western Cape Province (Chapter 4). This suggests that agents suitable of living in both climates or separate agents suited to each of the climates should be considered. Similarly, natural enemies found feeding on plants in the native distribution, that are genetically similar to those in the introduced distribution, are likely to have local adaptations for feeding on the invasive plant genotypes (Goolsby et al. 2006). In this case, insects which have been introduced and survived on plants genetically similar to plants growing in the invaded range in South Africa are likely to have local adaptations for feeding on the invasive plant haplotypes. The ability of the *S. terebinthifolia* hybrid population to inhabit niches that were not suitable to the original haplotypes (Mukherjee et al. 2012) and the reduced impact of the wasp on the hybrids in the USA (Geiger et al. 2011) highlights the importance of using agents that have been sourced from the correct plant genotype.

Three potential biological control agents other than *M. transvaalensis* have been shown to perform differently between the haplotypes and the hybrids. These include two thrips *Pseudophilothrips gandolfoloi* and *Pseudophilothrips ichini* (Thysanoptera: Phlaeothripidae) (Cuda et al. 2012) and psyllid *Calophya terebinthifolia* (Hemiptera: Calophyidae). In the native range *P. gandolfoi* is mostly associated with haplotypes C and D, and during testing on the haplotypes and hybrids in Florida, the species performed poorly. Whereas *Pseudophilothrip ichini*, which forms pit galls on the leaflets, collected from a haplotype A tree had high survival on the all haplotypes from Florida including the hybrid (Manrique et al. 2008). *Calophya terebinthifolia* was tested on five haplotypes (A, O, D, K, and M) and favoured haplotype A (Christ 2010). Agents suited to the correct haplotype should be prioritised. This study has taken some fundamental steps required for starting a biological control programme. Genotype and climatic matching have proven to be useful for prioritisation of regions in the native distribution where there should be focus on surveying biological control agents (Paterson et al. 2014).

5.1 Potential for biological control

Schinus terebinthifolia has a well-studied suite of natural enemies in its native range found in different climatic regions and on different haplotypes (Hight et al. 2002; Wheeler et al. 2016). A number of natural enemies have also been considered as potential biological control agents and they have undergone host range testing as well as studies to determine their performance on different haplotypes (Cuda et al. 2019). There are three agents that have been found to be suitable to haplotype A, namely: *Pseudophilothrips ichini*, *Calophya terebinthifoli* discussed abouve and *Episimus unguiculus* (Lepidoptera: Tortricidae) (Manrique et al. 2008: Cuda et al. 2019). *Episimus unguiculus* is a leaflet roller moth, which can completely defoliate small *S. terebinthifolia* plants (Manrique et al. 2008, Cuda et al. 2019). The moth was released in Hawaii, which only has haplotype A, and although the agent established, it was not sufficiently damaging (Hight et al. 2002). Cuda et al. (2019) suggest that the ineffectiveness may be due in part to biotic factors (parasitoides) unique to the Hawaii Island environment. These may not be the same in South Africa and the agent should therefore be considered. *Episimus unguiculus* was not released in Florida as it also oviposited and developed on the economically important *Pistacia* spp. and there is zero tolerance for agents that attack any crops or native plants under the current legislation in USA (Cuda et al. 2019). However, in the multiple-choice tests *E. unguiculus* exhibited a clear preference for *S. terebinthifolia* relative to the non-target plants used in the no-choice tests. Overall, the results of field observations during surveys in South America and Hawaii, and host range studies completed in Hawaii and Florida, showed that *E. unguiculus* is a narrow specialist on *S. terebinthifolia*, its natural host plant (Cuda et al. 2019). *Episimus unguiculus* may therefore be considered suitably host specific for release in South Africa.

Two other agents have been released in Hawaii which could be considered for South Africa, a seed-feeding beetle *Lithraeus atronotatus* (Coleoptera: Bruchidae) collected in Guapituba, Ribeirao Pires and Maua in Sao Paulo state (Krauss 1963), and a stem-galling moth, *Crasimorpha infuscata* (Lepidoptera: Gelechiidae) collected in Guapituba and Ribeirao Pires in Sao Paulo state, Salvador in Bahia, and Recreio dos Bandeirantes in Guanabara (Krauss, 1963). These regions of collections all have haplotype A plants, but at the time the collections were made researchers were not aware of the different *S. terebinthifolia* haplotypes, so the agents may have been collected off other haplotypes present in the area.

Another promising agent identified for possible release in both Hawaii and Florida, was the sawfly *Heteroperreyia hubrichi* (Hymenoptera: Pergidae). This agent was found to be damaging to the leaves of *S. terebinthifolia* and capable of complete defoliation of plants (Hight et al. 2002). Host specificity testing was conducted and the sawfly was considered safe to release in both Hawaii and Florida based on its specificity. The sawfly was not released as it was thought to be poisonous to livestock, as is the case with some other sawfly species (Hight et al. 2002). Further research has indicated that the sawfly is actually not sufficiently toxic to be a threat to live stock, but public opinion is now hampering the release of the agent (Dittrich et al. 2004; Wheeler et al. 2016).

5.2 Suggestions for management

Firstly, the combination of predictive models and new records of *S. terebinthifolia* in the Western Cape Province suggest the NEMBA regulations regarding *S. terebinthifolia* should be updated to include the Western Cape Province as a category 1b invasive species. The NEMBA regulations are updated regularly through the Department of Environmental Affairs.

Secondly, the invasion in Florida has been exacerbated by the hybridisation of the species and this should be avoided as far as possible in South Africa. Currently only a single haplotype has been found in South Africa and further importations of other haplotypes must be avoided at all costs.

Thirdly, *S. terebinthifolia* is known to be a problematic and aggressively invasive species; therefore, all appropriate measures need to be taken to reduce the extent and density of current invasive populations, and to reduce the likelihood of further invasions. Chemical and mechanical control measures for *S. terebinthifolia* are no longer viable options for reducing the overall population in South Africa but may still be useful in small-scale management initiatives. This is supported by evidence from the USA where chemical control has proved to be both expensive and ineffective on a landscape scale (Rodgers et al. 2012). It is suggested that biological control should be considered as a component of the management of *S. terebinthifolia* in South Africa and should be incorporated into the current management strategies. There has been some significant successes in managing weed populations through integrated control in South Africa, especially invasive trees (Hill & Coetzee 2008; Jones 2001; van Wilgen and Richardson 2014; Zachariades et al. 2017) and these expertise should be consulted before management options are considered.

Finally, biological control agents already known to be specific to *S. terebinthifolia* and capable of developing on haplotype A should be prioritised for importation into quarantine facilities in South Africa for further studies. Should these agents not be suitable for South Africa, it would then be important to select agents from regions compatible to South Africa population. In the native range coastal regions of Brazil, specifically regions between Florianopolis and Santos are regions where agents for haplotype A could be collected for biological control programmes in South Africa.

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