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AN ASSESSMENT OF THE CURRENT STATUS AND INVASIVE POTENTIAL OF *CROTALARIA AGATIFLORA* (CROTALARIEAE, FABACEAE) IN SOUTH AFRICA

By

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Summary

*Crotalaria agatiflora* is native to tropical East Africa (Tanzania, Ruanda, Burundi, Kenya and Ethiopia). In South Africa this plant was introduced as an ornamental garden shrub, and has since become invasive in many localities (savanna, grasslands, watercourses and forest margins). It is found along road verges, riparian areas, dump sites and abandoned sites in Limpopo, Gauteng, Mpumalanga, KwaZulu-Natal and one population in both the Eastern and Western Cape. This project aimed to see whether it poses a significant threat to the biodiversity and ecosystems of the country, so that information can be provided for developing a good management plan for clearing *C. agatiflora* in South Africa.

More than thirty populations of this species were recorded in the country and mapped. Five populations were selected to measure and count the number of individuals, analyse the soil to determine the seed bank and test the seeds for viability and germination ability. The potential future distribution of the species was modelled based on climatic suitability using MaxEnt to visualise the potential suitable range and ArcGIS to calculate (quantify) the expansion or reduction range of suitability between the current and future climate conditions. The Australian weed risk assessment model was also used to score (quantify) the invasiveness of this species. Furthermore, isozyme studies were done to determine whether a single or multiple introductions are responsible for the presence of this species in South Africa.

The soil-stored seed bank and records of occurrence show that *C. agatiflora* is a slow but persistent invader. Its seed coat dormancy and ability to re-sprout makes it very persistent. Seed germination tests showed 100% viability. A very high potential of invasiveness was indicated by the Australian weed risk assessment and the climate suitability models. The isozyme study showed no variation between populations indicating that the species has most likely spread from a single introduction. The scientific data obtained from this study show that *C. agatiflora* poses a significant threat to the flora of South Africa and that it should be controlled. The data will inform management plans of the Invasive Species Programme of the South African Biodiversity Institute.
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Chapter 1: Introduction

1.1 Background and problem identification

Invasive organisms have become a worldwide problem. They threaten natural ecosystems and replace indigenous and endemic species in large parts of the globe (Vitousek et al., 1996; Sakai et al., 2001). Several members of the legume genus *Crotalaria* L. (Fabaceae/Leguminosae) have become invasive in various parts of the world (Jaca et al., 2013). *Crotalaria agatiflora* Schweinf. is one example of such a species in South Africa and other parts of the world. It has invaded natural habitats, roadsides, wetlands and forest margins and seems to be spreading at an alarming rate. This study is designed to address this species as a potential threat to biodiversity in South Africa.

1.2 History and background of alien invasive plants

South Africa has an extremely rich biodiversity, with the richest temperate flora in the world (total of 20 456 species) occurring in the region (Germishuizen et al., 2006; Raimondo et al., 2009). Of these, 2 577 taxa are threatened with regional or global extinction. These threats are mainly through agriculture, urbanization, habitat loss and encroachment of alien invasive species (Raimondo et al., 2009). Currently, more than 660 plant species are known to be contributing to the widespread transformation of once pristine habitats in South Africa. Approximately 660 naturalized alien species are listed by the Southern African Plant Invader Atlas (SAPIA; Henderson, 2007).

In 1965 and 1974, Baker in his publications already discussed a few life history traits that are closely associated with weedy plant species and further proposed that species with many of these characteristics are more likely to be invasive than species with fewer traits. Traits that make species more invasive continue to be of great interest to the scientific community because of their predictive ability. Some of the traits mentioned by Baker (1965, 1974) that promoted weediness included the species’ ability to reproduce sexually and asexually, rapid growth from seedling to sexual maturity, adaptation to environmental stress and high tolerance to environmental heterogeneity (Sakai et al., 2001; Hastwell and Panetta, 2005). The
process of invasion and the relationship between variables were summarised by Sakai et al. (2001) and is shown as Figure 1.

Figure 1: There are several general steps in the invasion process and their relationship to management of invasive species (Sakai et al., 2001). These include transport, establishment, and spread of many invasive species as well as their effects, each with questions that may be relevant to and enhanced by studies in population biology, including studies of life history traits as well as consideration of genetic and evolutionary changes. Feedback may occur between many of these steps.
There is an enduring presumption in the science of invasive species that says that the abundance, density and frequency of introduction are directly proportional to the probability of a species establishing a population in a new region (Grevstad, 1999; Parker, 2001; Ahlroth et al., 2003; Cassey et al., 2005). It is also presumed that should introduction abundance, density and frequency decrease, the chances of a species establishing will also decrease. A number of studies have shown dissimilarities in how important different regions are as sources of invasions, but the driving force of these invasions is largely still to be explored e.g. specific traits, differences in opportunity (transport) or biotic and abiotic characteristics of invaded regions. Organisms can be exposed to different conditions that will affect the quality of the propagule. This mainly occurs during transportation processes; propagules may be severely damaged or stressed and these are unlikely to survive when compared to the ones that enjoyed luxurious transportation. Species traits that can influence the density and quality of propagules upon release include a host of characteristics that describe the life history, genetics, biology, and population abundance of a species should it survive initial release (Mgidi et al., 2007). Figure 1 as reported by Sakai, et al. (2001) illustrates the above-mentioned traits and other factors in the invasion process.

When human populations expand significantly habitat change is inevitable especially in protected areas (Wittenmeyer et al., 2008) and this has shown to have important consequences for conservation. There are many factors that interact to determine whether an organism can enter into and establish membership of a community. Prevention of invasive alien species is the most efficient and cost effective management option (Tu, 2009). In-depth knowledge on drivers of introductions, the numbers of species that could become invasive, and how this differs across various taxonomic groups therefore provides an essential basis for the formulation of appropriate practical policies and management options. Alien plant invasions have most commonly been shown to be highly related to human movement and density, as well as indigenous species richness (McDonald, 1986; McKinney, 2002; Pysěk et al., 2002). According to Spears et al. (2013) environmental variables such as climate, human factors i.e. roads and various other disturbance effects, and other specific characteristics are often comparatively less important and more variable across studies. In a study done in protected areas (mostly parks), the population
density is the most consistent predictor of numbers of alien and invasive species across both plants and animals (Spears et al., 20013). Even though there are other variables that can capture invasion drive, human population numbers is the better explanatory variable. The second most consistent significant term or variable in the explanatory models was data availability of occurrence points (Spears et al., 2013). Richardson et al. (2005) reported that variables that are associated with human activity seem to be more strongly correlated with alien species richness than with invasive species richness if indigenous species richness is ignored. Therefore indigenous species richness is important. Pysěk et al. (2010) believes the solution to future biological invasions is to combat the negative environmental consequences of human activities and the promotion of more sustainable growth.

1.3 Impact of invasive alien plants on the ecosystem and the extent of invasion

Le Maitre et al. (2000) estimated that approximately 10.1 million hectares of South Africa and Lesotho have been invaded by alien plants. Of the eight biomes found in South Africa (see Rutherford et al., 2006), the Western Cape Province, which largely comprises the Fynbos biome, is the most heavily invaded, particularly by woody shrubs and trees. This is followed by the Mpumalanga, KwaZulu-Natal and Limpopo Provinces. The largest total invader-transformed areas are those invaded by Melia azedarach L. and Pinus L. species (pines), followed by Acacia mearnsii De Wild., Prosopis L. species and Lantana camara L. These invasions deplete water resources (particularly true for woody invaders), affect delivery of ecosystem goods and services, over-utilize or alter natural resources (e.g. nitrogen addition), shift (often intensify) fire regimes, and affect sand movement and salt concentration (Richardson and Van Wilgen, 2004). Other effects include poisoning e.g. Bryophyllum delagoense (Eckl. and Zeyh.) Druce and Kalanchoe pinnata Lam.

It is well known that South Africa, like many other countries is no exception when it comes to hosting invasive species; some of which have spread across large areas and caused significant ecological and economic damage (Le Maitre et al., 2002; Le Maitre et al., 2011a).
One other important factor in the dynamics of invasion biology is Allee effects (Taylor and Hastings, 2005). Stephens et al. (1999) defines an Allee effect as “a positive relationship between any component of fitness of a species and either numbers or density of conspecifics” meaning that an individual that is subject to an Allee effect will suffer a decrease in some aspects of its fitness when conspecific density is low. Allee effects have been studied much lately because of their roles in species extinctions. Low density in the initial population or Allee effects of an invasive species can and is likely to offer an opportunity to alter the invasion dynamics in a particular region. This can be observed in terms of longer lag phases, slower spread and decreased probability of establishment (Taylor and Hastings, 2005). These changes in invasion dynamics can mislead one into underestimating the invasion risk posed by a particular invasive species. Ultimately these effects will determine optimal control strategies and costs.

1.4 Control and management of invasive species

Eradication and control of invasive plants are extremely costly. This may be done either through manual clearing, the use of chemicals (e.g. herbicides), or by the introduction of host-specific plant-feeding insects, mites and pathogens from the invaders’ country of origin, i.e. the plant’s natural enemy or enemies, into a new country where the plants have become problematic (biological control; Zimmermann et al., 2004). The price of clearing invasive species may vary depending on the density of the invasion and also on the species being cleared. It was estimated that South Africa allocated approximately R355 million to alien invasive clearing during the 2002/03 financial year (Marais et al., 2004). This highlights the importance of biological control as a relatively inexpensive and effective means of eradicating alien invasives as the biological control agents inflict damage and cause a decline in population densities, distribution and/or rates of spread of the problem plants, thus reducing the costs of other management practices (Zimmermann et al., 2004). Although most invasive plant species are widespread, there are still some species of invasive plants that have not spread very far since their initial introduction and only occur at a few localities within South Africa. According to Wilson et al. (2011) in the past most resources have been allocated to controlling and managing species that
were already widespread, however, there is a strong trend towards allocating more resources to reducing the invasion debt by preventing currently limited invaders from spreading, with the aim of reducing long-term expenditure on weed management. Although a bleak picture may seem to be painted about invasive alien plants, Geldenhuys (2013) suggests that invasive alien species may need to be dealt with in a different way especially when this high costs in controlling them are ineffective. The author further elaborates that those invasive plants in forest margins can be used as pioneer species by allowing them to form a nurse stand under which the indigenous trees can develop. The indigenous climax species can eventually replace the invasive species.

According to Pysek and Richardson (2010), detecting new invaders early, assessing and responding rapidly with a suitable management plan is deemed a good strategy in the management of invasive alien species. Management is crucial as the cost of controlling these invaders is directly proportional to the size of the invaded area (Rejmanek and Pitcairn, 2002). For some species eradication is considered more often as part of a proactive management plan to keep the invading plant species at controllable levels. This is done to also avoid future invasions by some species that are still in their lag phases (Wilson et al., 2011). Meyers et al. (1998) define eradication as the removal of all individuals of a species in an area, so that no re-colonisation is possible. This was further improved by stating that it is when all individuals and propagules of the invading species are permanently removed and reinvasion is highly unlikely; further spread of the species is prevented and positive changes in biodiversity and ecosystem services can occur (Myers et al., 2000; Wilson et al., 2013). Target species-specific eradication programmes are very important in managing invasive species, as different control methods work for different individual species (Simberloff, 2009). It is also of importance that these eradication and management programmes need to be based on ample prior knowledge of a species’ life cycle traits, for example the age of reproductive maturity to counteract further seed production, soil-stored seed bank to determine the extent of the control period, and the spread pathways to make sure that there is appropriate monitoring and control (Simberloff, 2003).

Cautionary measures or attempts to remove introduced species are taken because of the uncertainties of the potential invader and the risks it poses before they are
widespread. These precautionary measures are currently underway with many other species that are invading South Africa at a smaller scale or are still in the lag phase of invasion. Prior to these measures, the South African National Biodiversity Institute-Invasive Species programme (SANBI-ISP) carries out a detailed assessment of these species’ to determine their threatening status to the biodiversity and ecosystem services in a study similar to the one presented herein. Several factors are considered in determining whether a species should be eradicated or contained. Mgidi et al. (2007) lists: the biology of the species, the suitability of the environment and the characteristics of naturalised populations as some important factors to consider. The numbers of populations, long distance seed dispersal possibility, long lived seed banks, the degree or extents of invasion and the availability of effective control methods are some other important considerations for invasive plants (Simberloff, 2003).

Because seed banks are sometimes persistent and some plants reproduce with great vigour vegetatively, eradication efforts are often unsuccessful (Richardson and Kluge, 2008). However, it has been shown that with years of good monitoring and follow up efforts after the initial attempt of eradication many populations have been successfully removed (Regan et al., 2006). In South Africa, the common mechanical method of control of alien plants is to cut them down and apply herbicide to the stumps but this is not successful or practical for all plants. According to Rejmánek and Pitcairn (2002), only species with relatively small populations are considered practical to be eradicated as the cost of eradicating a species is directly proportional to the infestation sizes until removal of species is no longer a useful management strategy. It is reported that detecting an invasive alien species early and responding timeously with good or suitable measures of control increases the chances of a successful eradication. Byers et al. (2002) proposes an early detection and rapid response approach to managing invasive species as a highly cost-effective and good management strategy.

Successful eradications are to an extent dependant on how early the invasive species is detected and immediate or rapid response with fitting control measures. The question of whether eradication should or should not be attempted is crucial soon after a potential invader has been detected in a given area, as many eradication attempts are unsuccessful, mainly for plants. These failed attempts are
likely due to insufficient resources and efforts, huge number and infestation sizes, long-lived seed banks and low detectability which often results in failure to contain species (Panetta et al., 2011; More et al., 2011). Before a decision can be made about whether eradicating a species is feasible or not and prioritising it for eradication, sufficient population-level information must be available (Cunningham et al., 2004).

According to Panetta and Lawes (2005) an assessment of potential options for controlling invasive species depends on accurate delimitation and on evaluation of the threat posed by a species to native ecosystems. Species that are invasive may exist as one or several populations arising from either a single or multiple introductions. Eradication can be attempted after the full extent of the invasion is known because the amount of resources needed, the optimal control strategies and the feasibility of eradication can only be determined after an extensive study of the invaders life history traits (Lawes and Panetta, 2004). According to Wainger and King (2001) when available funds and resources are limited for the invasive species management, species can be prioritised based on the level of risks or threats they pose. Risks can be assessed using the Australian weed risk assessment protocol. This method has been used by the biosecurity organisations throughout Australia and New Zealand (Pheloung et al., 1999) and has been proven to be applicable in other parts of the world by some authors (Gordon et al., 2008; Dawson et al., 2009; Andreu and Vilá, 2010).

During control activities management can further evaluate its plans and improve their methods by means of observation and data collecting i.e. how effective the control treatments are and species search protocols (Cacho et al., 2006). Detecting a species is another crucial factor influencing the amount of effort required to search and locate all individuals especially at juvenile stages. If species are detected at low levels, they will require greater search efforts such as a high number and level of expertise of surveyors and an extended time will be required to complete the search (Gerrard et al., 2008; Tyre et al., 2003). Many of these factors can be used in eradication plans to ensure optimal and cost-effective management plans.

South Africa has policies and a legislative structure in place to protect our ecosystems and biodiversity. This was done to reduce the negative impacts of non-
native plants in the country. Plants are listed in different categories according to their invasive extent or potential invasiveness. These categories serve as a guideline for nurseries and landowners to control the spreading of these invasive populations. The Conservation of Agricultural Resources Act (CARA) and the National Environmental Management Biodiversity Act (NEM:BA) places non-native plant species in categories according to their ecological and economic threats they pose and each category clearly states how the species under that category is to be managed. Under the proposed NEM:BA regulations, category 1a comprises invasive species with small populations that require compulsory control. Species in category 1a are targeted by the Invasive Species Programme of the South African National Biodiversity Institute (SANBI-ISP) for eradication.

1.5 Southern African Plant Invaders Atlas (SAPIA) and world database as helpful tools in locating invasive plants locally and globally.

The Southern African Plant Invaders Atlas (SAPIA) is a database of all known invading plant species in South Africa with records of localities that are encroached by alien invasive plants. To study *C. agatiflora*, basic knowledge about the species had to be known; where the species originated from and how it came into South Africa, as well as the areas that are invaded by this plant. SAPIA, herbarium records and the Global Biodiversity Information Facility (GBIF) were good starting points. A search for *C. agatiflora* was done on the two databases to locate populations of this species in South Africa and globally. Herbarium records were studied to trace back the earliest record of this plant in South Africa. All the records of *C. agatiflora* from the Pretoria National Herbarium (PRE) and Bews Herbarium (NU) were studied. In addition, Rod Randall’s list of invading plants (African Centre for DNA Barcoding, 2015) was consulted but surprisingly *C. agatiflora* was not amongst the invasive Crotalaria species on the list. Other lists that were considered included Dr Tony Rebelo’s (SANBI) list of additional invasive plants in the Western Cape region as taken from the African Centre for DNA Barcoding (ACDB.co.za), South African plants invasive elsewhere in the world also by Rod Randall and listing of alien species and invasive species taken from the notice under chapter 5 of the National Environmental
Management: Biodiversity act of the Department of Environmental Affairs (DEA) and Tourism (DEA, 2014).

It is a major challenge to predict which species are likely to be more successful as invaders than the others and which ones are likely to become invasive. According to Goodwin et al. (1999) two correlates are normally used in assessing risks, i.e. the degree or extent of invasiveness of a plant species anywhere else in the world and whether other plant species of the same type can invade a certain area. There are many factors to consider in determining the invasiveness or extent of invasiveness of a plant species. For example; the time when the plant was first introduced to a particular habitat and the time it started showing invasive signs ("residence time") and the potential range are also key factors (Wilson et al., 2007). Importantly, some populations after a decade or more would still show no signs of invasiveness, a stage referred to by some as “lag phase” which exhibits very slow/limited spread of the population (Fig.2). This characteristic is also illustrated in Fig. 1, as reported by Sakai et al. (2001). Some authors report that many invasive species can become widespread and take over large areas provided there is sufficient time to adapt and naturalise (Caley et al., 2008), and that the species is climatically suitable to the large area (Rouget et al., 2004); such widespread species are deemed to be very difficult to control and manage.

![population growth curve](image)

Figure 2: A population growth curve (Fischer, 2004) showing a pattern followed by most invasive species across the world. A lag phase is observed initially, an increase in populations and then exponential growth.
The spread of a species may be limited by ecological and probability factors which might be the reason for the extinction of these propagules or this might simply be a part of the exponential growth phase (Sakai et al., 2001). An expectation of invasion in the future emanates from this time delay; that species that are currently not invasive will become invasive in the future. This is also termed an “invasion debt” or simply the lag phase of the conservation crisis that follows (Essl et al., 2011). Data of various invasive species from 28 countries in Europe was examined in this study and they caution that “the seeds of future invasion problems have already been sown”, simply meaning that the effects of the invasive species observed today is the result of previous activity and invasions from many years ago. With that they make a call for increased control efforts and reduction in transportation of these species to new habitats as they believe global trade taking place today is driving invasions for many years to come (Essl et al., 2011). Some introductions are due to accidental seed transfer that is associated with the ever accumulating human and cargo movements globally (Nathan et al., 2008; Wilson et al., 2009). In most instances a species is introduced multiple times or frequently with a high number of seeds; this is considered an important factor in determining the invasive potential of the species (Lockwood et al., 2005).

1.6 Crotalaria as an invasive genus in the world

Legumes (whether trees or shrubs) are known to be very successful invaders, with 122 species invading different regions in the world (Rejmanek and Richardson, 2011). Legumes are very popular as garden plants because of their spectacular flowers, endurance and ability to flourish in nutrient poor soils. It is these reasons and the history of long seed dormancy in many species in this family that makes it one of the most disreputable/notorious contributors to the naturalised flora of the world (Geerts et al., 2013). The majority of invasive plant species are to a great extent distributed mainly because of human usage of that particular species (Lavergne and Molofsky, 2004; Wilson et al., 2007; Grauer et al., 2008; Wilson et al., 2011; Procheş et al., 2012). All around the world, legumes have been introduced for various uses, mostly in agriculture and horticulture. According to Graham and Vance (2003) legumes are the second most important agricultural plant family after grasses
because of their ability to fix nitrogen, resist drought and the large number of species that are edible.

The legume genus *Crotalaria*, also known as rattle pods or klapper bossie, are very common in tropical countries. About 702 species belong to the genus *Crotalaria* and they are widely distributed in several other continents (Jaca et al., 2013; Le Roux et al., 2013) with tropical or subtropical climates. In total 500 species were recognized by Polhill (1982) in Africa and Madagascar. This large genus is like other legumes that fix nitrogen; they are very useful as fodder and green manure but many of them are poisonous to livestock. In Southern Africa there are about 60 indigenous *Crotalaria* species and, this could mean an increased impact on biodiversity and ecosystem services if there is hybridisation (Polhill, 1982). There are 15 species in the genus *Crotalaria* recorded by Rod Randall as invasive in the world (African Centre for DNA Barcoding, 2015). The invasive species are *Crotalaria burkeana* Benth., *Crotalaria distans* Benth., *Crotalaria dura* J. M. Wood & M. S. Evans, *Crotalaria globifera* E. Mey., *Crotalaria laburnifolia* L. subsp. *australis* (Baker f.) Polhill, *Crotalaria lanceolata* E.Mey., *Crotalaria lotoides* Benth., *Crotalaria natalitia* Meisn., *Crotalaria pallida* Ait. var. *obovata* (G. Don) Polhill, *Crotalaria pallida* Ait. var. *pallida*, *Crotalaria recta* Steud. ex A. Rich., *Crotalaria spartioides* DC., *Crotalaria sphaerocarpa* Perr. ex DC., *Crotalaria sphaerocarpa* subsp. *sphaerocarpa*, *Crotalaria virgulata* Klotzsch subsp. *grantiana* (Harv.) Polhill.

1.7 *Crotalaria agatiflora* in South Africa

*Crotalaria agatiflora* (known as voëltjiebos or canary bird bush) is native to tropical East Africa and occurs mainly in Tanzania and Kenya (Polhill, 1982). In South Africa it has been reported to be present in the Gauteng, North West, Limpopo, Mpumalanga, and KwaZulu-Natal Provinces (SAPIA database), but its status as an invasive plant is still unknown and in need of assessment. *Crotalaria agatiflora* was originally imported as an ornamental plant and initially proposed as a category 1a invader under National Environmental Management: Biodiversity Act (NEM:BA), meaning that its import is banned and that it requires compulsory control in the country (Bromilow, 2010). Some scientists within the Invasive Species Programme of the South African National Biodiversity Institute suspect that this species might have to be moved to category 1b as its phases of invasion seems to have advanced. The exact effects of this species in South Africa are, however, not yet clearly understood.

*Crotalaria agatiflora* has not yet been recognized as invasive in the literature, but from field observations one may hypothesize that it has the potential to be invasive.
The species is naturalised in Australia and showing signs of weediness in New Zealand. In Gauteng it is showing some signs of weediness along road-sides, water courses in grassland and savannah biomes, and also on forest margins. It is believed to have been introduced into the country as an ornamental plant with the earliest records of the species housed in the Pretoria National Herbarium (PRE) dating back to 1921 and 1960. There are currently 57 records of locations of the species in South Africa (SAPIA, database). There used to be uncertainty about which subspecies of *C. agatiflora* [subspecies *agatiflora* or subspecies *imperialis* (Taub.) Polhill] has been introduced to South Africa. This will be verified by comparing authentic herbarium specimens and through collaboration with researchers from the country of origin. Its invasiveness is not documented in the literature, but from sight observations one can deduce that this species has great potential to be invasive due to its abundance along roadsides. *Crotalaria agatiflora* was not included in the list of 15 invasive species (African Centre for DNA Barcoding, 2015).

*Crotalaria agatiflora* is beginning to spread at an alarming rate, with the most number of populations recorded in Gauteng and Mpumalanga. Most populations of this species are relatively small in size. It is possible that insects or biocontrol agents might have been the reason why *C. agatiflora* in South Africa has remained unnoticed or in the lag phase for a period of about 100 years.

Attempts to see if *C. agatiflora* can be eradicated even at this stage of its risk assessment shows how time is of great importance in early detection and rapid response. In the Gauteng, Mpumalanga and North West Provinces of South Africa, some level of success has been achieved in an attempt to eradicate it but more labour, resources and energy is required. Assessment of this species should provide information in order to decide whether eradication, containment or biological control of *C. agatiflora* is feasible.

1.8 Invasive Species Programme (ISP) mandate, failed attempts and successes

The Invasive Species Programme of the South African National Biodiversity Institute (SANBI-ISP) has drawn a mandate of which this project is aligned to fulfil. The
mandate attempts to explain the work flow of the Invasive Species Programme (ISP); that is to detect new invaders in South Africa by monitoring and managing records of new localities of naturalisation, evaluating species in detail to be able to offer enough information to decide whether regulating the species is necessary and if so, in which way. Data is needed for planning eradication procedures by estimating the cost and practicability of removal of every single individual of the species including all seeds and vegetative propagules or revising the listing of the species legislatively (Wilson et al., 2013).

1.9 Objectives and key questions

1.9.1 Determine the present distribution of *Crotalaria agatiflora* in South Africa

The Southern African Plant Invaders Atlas database (SAPIA) and the Global Biodiversity Information Facility (GBIF) and herbarium records are good sources for providing occurrence data of many species whether invasive or not. Distribution data are valuable in assessing invasive species as this would serve as a guide for determining their current status and developing management plans.

According to Mortensen et al. (2009) habitat suitability and corridors that aid dispersal strongly influence the spread and abundance of invasive species. Different habitat types (road sides, wetlands and intact areas) may yield different types of results in terms of habitat invasibility. Landscape features or habitat differences are important to consider when drafting policies and options for management aimed at mitigating invasive plant abundance and distribution (Mortensen et al., 2009). Despite the research that has gone into invasive species, it is very difficult to point out traits that are consistent in predicting invasiveness; this may be largely because different traits favour invasiveness in different habitats (Pysek, 1998; Alpert et al., 2000). An interesting report by Williamson and Fitter (1996) suggests that only 0.1% of all plants introduced by humans outside their native ranges become invasive. It is known by now that prevention of all introductions is not feasible, but knowing which species to look out for in future might provide the possibility of reducing the number of invasive species. Recording and mapping all known current *C. agatiflora* populations can aid in showing the true spread of the species and the seriousness of this invasion.
*Crotalaria agatiflora*, like most invasive species, originate from natural habitats far removed from the point of introduction. Horticulture is central to the introduction of this species. Approximately 60% of invasive species in the world, including those in South Africa, are introduced horticulturally (Reichard and White, 2001). Management and conservation practices are responsible for introducing about 30% of invasive species in the world and accidental introductions account for nearly 10% (Boy and Witt, 2013). Of all species introduced, only about 15% ever escape cultivation, and of this 15% only about 1% ever become a problem in the wild (Boy and Witt, 2013). The process that leads to a plant becoming an invasive species, i.e. Cultivation – Escape – Naturalization – Invasion, may take over 100 years to complete (Sakai et al., 2001).

*Crotalaria agatiflora* populations occur on different scales from one isolated plant on a roadside or encroaching onto a natural habitat to as many as 500 or more individuals. Generally the populations are relatively small; but some large populations do exist along roadsides, some river courses and wetlands in South Africa. These plants have been reported by the Southern African Plant Invaders Atlas (SAPIA) in different climatic regions of South Africa from Mediterranean, warm semi-arid to warm and cool subtropical climates, excluding dry hot and arid regions. *Crotalaria agatiflora* growth and expansion seems to favour different habitats with different climates in South Africa and this makes it highly competitive to indigenous species.

1.9.2 Investigate the size and growth characteristics of existing populations of *Crotalaria agatiflora*

There are many characteristics that make invasive species like *C. agatiflora* successful or unsuccessful in a new environment (Binggelli, 1996).

Bruchid beetles are known to be predatory species on seeds (Sales et al., 2000; Fox et al., 2012). These are coleopteran insects from the family Bruchidae and have since antiquity been known to be associated with legumes through co-evolutionary processes. These processes have allowed weevils to flourish on feeding from seeds of these leguminous plants irrespective of toxic compounds produced. Weevils have
evolved to take advantage of the nutritional value of legume seeds (Sales et al., 2000). However, Bruchids are also important parasites of these seeds as they might have an unpredictable effect on germination. This effect can be two-fold; either scarification of the seed for germination or utterly destroy the embryo and deplete seed resources (Fox et al., 2012). These beetles have been identified on C. agatiflora in several populations across the country where they puncture holes in the pods to feed on the seeds. Could weevils be the reason why the spread of this species has remained in a lag phase for a long time? Or are there perhaps more reasons why C. agatiflora remained part of category 1a (considered of limited importance as an invader) taxa of the National Environmental Management: Biodiversity Act (NEM:BA) for such a long time?

Biological invasions have shown to be problematic mainly because they are random and occur in no particular order and often involve small populations that can survive rapid habitat transitions. Baker and Stebbins (1965) were influential in their work on evolutionary dynamics or characteristics of invasions, i.e. how genetic makeup might impact on the ability to invade. Importance of genetic attributes for invasion success is backed by evidence such as genetic variance, genetic background, hybridisation, genetic trade-off actions of small numbers of genes and possibly genomic rearrangements. Lee et al. (2002) reported on the possibility that invasion success could be facilitated by the presence of a genetic substrate or substance in source populations upon which natural selection could act.

Disturbance maybe another factor adding to population growth characteristics. It appears that disturbance is important early in the invasion process because it is able to produce an unoccupied niche for alien plants to fill (Masters and Sheley, 2001). This might be mechanical, edaphic and/or fire disturbances, but are able to significantly influence the spread of species. Carvalho et al. (2010) reports that disturbance may promote invader success by opening opportunistic windows to alter soil enemies and mutualistic effects. Crotalaria agatiflora appears to be affected by disturbance, especially fire. Although this thesis does not cover much detail on the fire disturbance regimes of this species or legumes in general, a few populations have been observed over time. When one compares populations from before and after fire exposure, re-sprouting and seedling growth are noticeable (Jaca et al., 2013).
1.9.3 Quantify the number of seeds in the soil-stored seed bank

Seed banks have been proven to drive plant invasions around the world, particularly legumes. *Crotalaria agatiflora* is now showing signs of weediness and is widespread around the country in somewhat dense and extensive populations found on roadsides, river edges, grasslands and forest margins. The seed bank below a mature stand and re-sprouting of most legumes species prove to be very important in their spread. Abundant seed produced by *C. agatiflora* leads to the establishment of large and very persistent seed banks which seems to be the invasion characteristic of many legume invaders. However, the seed bank varies between sizes of populations as *C. agatiflora* populations range between five plants to five thousand plants including seedlings. This species produces greenish-purple pods that are inflated, ca. 100 mm long with 24 to 26 seeds in a single pod which rattle and are loose in a dry pod. The history of dispersal, population growth characteristics and seed bank of *C. agatiflora* are not well-studied and this study offers a chance for proper investigation.

1.9.4 Gain an understanding of the significance of the threat posed by this species on the rich biodiversity of South Africa

This is one of the fundamental questions posed in risk assessments of invasive species; whether a species is going to show harmful effects on the South African biodiversity, ecosystem functioning and services. An invasive alien species is one that has been introduced into a non-native range, either through deliberate or unintentional means, and once established in the new range can spread in such a way that it threatens habitats, ecosystems or even their economics (Cook et al., 2007). *Crotalaria agatiflora* is suspected to be poisonous to livestock and encroaching on native pristine habitats replacing or outgrowing indigenous vegetation. Control or eradication attempts of *C. agatiflora* are already in full effect as this plant is being assessed for more effective or suitable control methods such as biocontrol.
1.9.5 Investigate potential future distributions of this species using predictive modelling techniques

*Crotalaria agatiflora* is native to tropical East Africa. It has now spread across many parts of the world through various means of transportation, especially for use as an ornamental garden plant. Current spread and growth characteristics of invasive species have been studied but traditionally it has not been easy to study these species at a large scale. However, the recent increase in species distribution habitat modelling has made this process more practical (Austin, 2007). Modelling of species distributions examines relationships between species occurrence and the environment (Segurado and Araújo, 2004; Austin, 2007). Suitable environmental maps can be produced from these analyses which can also deduce potential future distributions of species. Therefore this tool can be regarded as effective for some aspects of invasive species (Peterson and Vieglais, 2001; Peterson, 2003; Iguchi et al., 2004). The resulting analyses from such tools can be utilised in invasive species programmes for management of areas that might show susceptibility to establishment of particular species. Modelling habitat suitability also helps land managers to develop hands-on management approaches because these tools also aid in identifying the environmental variables that are most important in the species spread.

Maximum Entropy (MaxEnt) is a high performing species distribution modelling method (SDM) that uses species occurrence and environmental data for predicting potential habitat suitability (Philips et al., 2006; Elith et al., 2006). The Maximum Entropy software is regarded as one of the easiest machine-learning algorithms that matches presence locations to different environmental variables at those localities and then across the study area to generate predictions in areas where the species has not yet been recorded, but might be able to invade in future. MaxEnt has proved to work well for mapping invasive species (Ward, 2007; Stohlgren, et al., 2010; Jarnevich and Reynolds, 2011) because climatic similarities between native and target regions is considered a requirement for successful invasions (Panetta and Mitchell, 1991; Mack, 1996; Welk et al., 2002; Robertson et al., 2004; Thuiller et al., 2005; Richardson and Thuiller, 2007). Matching of the climatic conditions plays an important role in predicting climatic suitability of species habitats, however, this is not achieved single-handedly but biotic factors and other aspects also need to be
considered (Thuiller et al., 2006; Stohlgren and Schnase, 2006). Occurrence data of the species in the native range can be used to model their climatic niche and areas that are most likely for establishment of propagules (Kolar and Lodge, 2001; Elith, 2014).

1.9.6 Calculate the cost and efficacy involved in clearing *C. agatiflora* and make recommendations on how to best manage this species in South Africa

Studies done across the world have demonstrated that environmental-economic evaluation techniques can contribute towards understanding the impact of invasions and development of regulations and recommendations for management on costs relating to alien invasive plants (De Lange and Van Wilgen, 2010). Some of these studies have managed to successfully show that the environmental-economic approach to dealing with alien species can deliver enormous cost benefits by reducing expenditure (De Lange and Van Wilgen, 2010). About R6.5 billion was estimated to have been lost every year because of invasions, but had control not been practised the estimated amount would have escalated to R41.7 billion, which means that R35.2 billion has been saved annually through application of different control methods (Van Wilgen and De Lange, 2011). It has been said that effective management of plant invasions combines different control options that may include chemical, mechanical, biological control as well as ecosystem rehabilitation. Therefore, environmental managers have come to realise the need for effective control programmes that can counter-act the effects of invasions. Study programmes are funded by environmental managers to determine causes of such impacts and factors underlying these impacts as the success or failure to manage future invasions rely on the results of such studies (Van Wilgen et al., 2001). Recommendations from this thesis may determine the success or failure to contain or eradicate *C. agatiflora* in South Africa.
1.10 Hypotheses

- *Crotalaria agatiflora* is a slow but persistent and potentially aggressive invader
- The invasive category of *Crotalaria agatiflora* (currently 1a) and the policy for its control should be reconsidered

1.11 Concluding remarks

Field observations indicated that this species hinder growth of other species, reduce biodiversity and alter ecosystems where it invades (roadsides, grasslands, riverbanks and forest margins). It remains to be seen whether containment or eradication at this point is the most feasible method.

Literature reviewed above suggests a whole range of methods to determine the status of a species as invasive or not. One can deduce that *C. agatiflora* indeed possesses attributes which will enable it to encroach on natural habitat. However, a detailed study of the risks this species poses was considered necessary to determine its invasive potential in South Africa. Different methodologies were employed to arrive at a certain conclusion regarding it as an invader and a threat to biodiversity and ecosystems.
Chapter 2: Materials and Methods

2.1 Study sites

As guidance for field work a field survey form was developed to use in the field whenever populations were visited, so that similar characters and variables could be observed consistently across different populations. Five populations of *C. agatiflora* were analysed across three provinces (Gauteng, Mpumalanga, KwaZulu-Natal) in South Africa (Fig. 3). Sites or populations studied (Fig. 3.) are (1) Colbyn Valley wetland, (2) Faerie Glen Nature Reserve, (3) Pinehaven, (4) Howick, and (5) Lowveld Botanical Gardens. Similar variables were observed and recorded at all locations. These include grid references of localities, altitude, distance from the road (to determine if it is a disturbance weed), sizes of populations (to monitor growth of these populations over time), aspect/slope, soil type, population structure [seedlings, young plants (pre-reproductive), young adult plants (flowering), mature plants, old plants (multi-stemmed, survived fire)] and evidence of root nodules. Flower visitors/pollinators were observed and the effects of fire were assessed. Soil samples, herbarium specimens, pods and seeds and leaf material for DNA studies were collected. Criteria used to allocate each plant a category was according to the shape of plants and in all the sites evidence of fire was noticeable from burnt surrounding. The height of the plant and growth form was measured to compare with the species in the native range.
Figure 3A: Colbyn Valley wetland population of *Crotalaria agatiflora* in Pretoria, Gauteng Province. (Figure: Google Earth).

Figure 3B: Faerie Glen Nature Reserve population of *Crotalaria agatiflora* in Pretoria, Gauteng Province (Figure: Google Earth).
Figure 3C: Pinehaven population of *Crotalaria agatiflora* alongside the N14 in Mogale City, Johannesburg, Gauteng Province. (Figure: Google Earth).

Figure 3D: Howick population of *Crotalaria agatiflora*, on Boston Road off the N3 north of Pietermaritzburg in KwaZulu-Natal. (Figure: Google Earth).
2.2 Desktop study of the invasive potential posed by *Crotalaria agatiflora*

Members of the genus *Crotalaria* are known to be invasive throughout the world. This group of plants was reviewed by doing a literature study and by searching for *Crotalaria* species on the International Plant Name Index (IPNI) to record all species names. The names were used to search the Global Biodiversity Information Facility (GBIF) database for records in the native range of each species and if it is naturalised or invading elsewhere in the world. The number of species from IPNI that also appears on GBIF could be determined, as well as the number of species that appeared outside their native ranges.

Figure 3E: Lowveld Botanical Gardens population of *Crotalaria agatiflora* in Nelspruit, Mpumalanga Province. (Figure: Google Earth).
2.3 Population dynamics

2.3.1 One transect through each of five populations to collect soil samples for determining some population dynamics.

To determine the soil-stored seed bank and collect soil from below the canopy of the population, a transect method was used which required a tape measure to be run across the population (Fig. 4A). A 30 m tape was used and extended to 4 m outside of the population on each end to see if the seeds spread further outside of the population or if they are just concentrated below the canopy. The orientation of the tape/transect was standardised (from east to west) in all five populations and run only once across, starting the tape 4 m outside of the population on the eastern side and ending the tape 4 m outside the population on the west. Soil samples were collected every 2 m along the tape measure, digging to a depth of approximately 10 cm at an area of 25 cm². The number of soil samples collected depended on the size of the population. The soil samples collected were sieved with 8 mm and 4 mm core sieves to recover all the seed present in the soil. The objective in this sampling design was to capture and record the number of seeds that were able to spread/disperse laterally. Some of these seeds were used for viability and germination testing. This procedure was repeated for all five populations.

Figure 4A: Transect method used when collecting soil samples for seed bank analysis. 25 cm x 25 cm x 10 cm holes that are 2 m apart were dug to collect soil samples from an easterly to a westerly direction in all five of the sampled populations of Crotalaria agatiflora.
2.3.2 Sample quadrants with three plants in each of four populations for flower and fruit counting

To determine the seed bank and collect soil from individuals within populations, a quadrant sampling method was also used. Four populations (Gauteng only) were selected to be used in this study because of large quantities of soil collected for analysis, namely the Colbyn, Faerie Glen, Pinehaven and Voortrekkerhoogte populations. For each population, three mature individuals were selected for study. Four quadrants of 2 m² each were placed around each one of the three large, mature individuals that were non-randomly chosen to represent that population (Fig. 4C). The aim of this sampling design was to estimate the maximum number in seeds in the seed soil bank.

Before soil samples were collected, the chosen plants were subjected to a detailed study of flowering and fruiting. Racemes and flowers were counted so as to follow up on how many of the flowers were pollinated and how many actually produced pods with viable seeds. Black clamps (cable ties) were used to mark the racemes on the plants chosen for this experiment (Fig. 4B). The aim was to calculate or estimate the number of seeds that reach the soil relative to the number of racemes, flowers and pods produced per plant.

Figure 4B: (A) Crotalaria agatiflora inflorescence with undeveloped pods and dead flowers. A black clamp (cable-tie) is attached to the inflorence as a marker to allow follow up and monitoring studies. (B) C. agatiflora inflorescence with healthy developed pods. A black clamp is also visible to track those inflorescences that are being monitored for development. Photos: Thabang Phago.
Soil samples were then collected to a depth of 10 cm within each quadrant. Soil was sieved and seeds were counted to estimate the number that reached the soil after being explosively ejected from the pods. The seeds were also used to test for viability with the aim of determining the reproductive capacity of the species.

Figure 4C: Sample quadrant (2 m²) under canopy of *Crotalaria agatiflora* in Colbyn, Pretoria (one of four from around the same individual plant). Soil samples were collected within the quadrant down to a depth of 10 cm. Photos: Thabang Phago.

2.3.3 Soil analyses on collected soil after extraction of the seed

The extent to which soil type may be related to the spread of *C. agatiflora* in South Africa was investigated. The main question was whether the soil type may limit the spread of the species. The Agricultural Research Council-Institute for Soil, Climate and Water (ARC-ISCW) released data in 2004 to indicate soil patterns in the different parts of the country. Soil was analyzed for different minerals across the five populations in the three provinces to see if *C. agatiflora* prefers a certain soil type. One sample was taken from the middle of the population and the others from the western and eastern boundaries of the population. For this experiment different soil parameters and nutrients available in the soil where *C. agatiflora* invades were studied to see if there was any relation between soil structure, soil nutrients and the observed distribution pattern. The soil samples were tested for percentage of organic
carbon, phosphorus, potassium, sodium, calcium, magnesium and in addition to the pH, particle size and soil resistance. I did X-Ray Diffraction (XRD; Fig. 5) and X-Ray Fluorescence (XRF) at the University of Johannesburg to determine the mineral composition of the soil.

The following analyses were done by the Agricultural Research Council Institute for Soil, Climate and Water (ARC-ISCW). Soil texture was studied by determining particle sizes. For organic carbon determination, the Walkley-Black method (Walkley, 1947) was used. For extraction of N-NH4, the KCl method (Wada, 1987) was utilised and the results analysed using an auto analyser. P was extracted using the Bray 1 method (Bray and Kurtz, 1945) and was analysed by the same auto analyser. Ca, Mg, K and Na were extracted with ammonium acetate and analysed by Inductively Coupled Plasma: Optical Emission Spectrometry (ICP:OES) (Hou and Jones, 2000). These soil analyses were done to see if C. agatiflora prefers a certain type of soil or not and to determine if there were any similarities or differences in the soil composition of the different populations. Inductively Coupled Plasma/Optical Emission Spectrometry (ICP: OES) is an excellent tool used in the determination of metals in a variety of different samples. Trace elements in many different samples are analysed using this method; it is applicable in different areas of science such as agricultural, food, biological, clinical, geological, and environmental applications (Hou and Jones, 2000).
2.3.4 Pollinator exclusion experiment in three populations

Pollinator exclusion experiments were conducted in three different populations, namely Colbyn, Faerie Glen and Voortrekkerhoogte. Inflorescences of *C. agatiflora* were covered with sleeves of porous material that could let in air and the sun but not limit any environmental influences except to keep out all flower visitors. Bees and birds were successfully kept out by the sleeves covering the racemes to ensure that no pollination could take place. Plants that were chosen for this experiment had to bear flowers that were still closed to show evidence of no visitation to the flower yet by birds as the obvious pollinators of this species.

2.3.5 Flower and seed counts in five populations

In all five populations, plants representative of the population were monitored for floral and seed counts to relate the number of flowers to the number of fruits produced. In this way I attempted to approximate the possible number of seeds that can be produced.

2.3.6 Tests of seed viability and longevity

A tetrazolium (TZ) solution was prepared to do seed viability tests. In TZ testing, as it is commonly known, sodium phosphate and potassium phosphate were prepared according to the following recipe (Peters, 2000):
Solution 1 - Dissolve 9.078 grams KH$_2$PO$_4$ in 1000 ml water.

Solution 2 - Dissolve 9.472 grams Na$_2$HPO$_4$ in 1000 ml water.

Two parts of solution 1 were mixed with three parts of solution 2. The final solution was produced by adjusting the pH to 7.5 using 2, 3, 5 triphenyl-tetrazolium chloride (TTC).

Seeds collected from the soil were divided into two batches and tested in two cycles. The first batch of seeds (tested six to 12 weeks after collection) was tested for viability and germination. In the same way, the second batch of seeds (tested after one year from the day of collection) was tested. These tests were done to determine viability and how long the seeds can remain viable. The seeds were mechanically scarified and imbibed in lukewarm water overnight. The following day, seeds were exposed to the tetrazolium solution and incubated at 35°C for 24 hours. Some seeds were not scarified to test for dormancy. *Vicia faba* L. seeds from the same family of legumes were used as positive control and non-viable *Xanthocercis zambesiaca* Baker seed were used as negative control.

2.3.7 Climatic modelling

Climate data of the current and future scenarios were extracted from worldclim ([http://www.worldclim.org](http://www.worldclim.org)), representing interpolated climate records from the year 1950 to the year 2000 at a resolution of 2.5 minutes (Hijmans et al., 2005). Initially 19 bioclimatic variables plus altitude as an additional variable were tested as potential predictors. Bioclimatic variables were downscaled to best predictor variables deduced from the results of the first MaxEnt run; these MaxEnt results are able to show which variable best predicts the species distribution. For the future scenario, only the best predictors of the 20 bio-variables were chosen and used for future climatic predictions at 2.5 minutes resolution for the year 2080. Climate scenarios (current and future) were obtained using Commonwealth Scientific and Industrial Research Organisation (CSIRO-mk30) and Canadian Centre for Climate Modelling and Analysis (CCCMA-GCM3) with general circulation model and the SRES A1B carbon emission scenario which assumes maximum energy.
MaxEnt version 3.3.2 software was used as a programme of choice for species distribution modelling (Phillips et al., 2006) to generate the bioclimatic envelope describing the current distribution range of *C. agatiflora*. The quality of the species distribution model (SDM) can be demonstrated by calculating the area under curve (AUC) value. An AUC value that is >0.9 indicates that the prediction is “very good” and highly probable, while an AUC >0.8–0.9 is deemed “good” and an AUC >0.7–0.8 is “useful”; this process is known as discrimination (see example below, Fig. 6A). All occurrence data of *C. agatiflora* worldwide was included to give a good predictive range versus biased. Jacknife statistics were all above 0.8, indicating a good performance by the model for this particular species (see example below, Fig. 6B). Records of occurrence were randomly split into training (75%) and test samples (25%). To reduce the impact of model over fitting, no duplicate records were included in grid cells with the same presence record. Outputs of the species distribution model follows a logistic distribution, ranging from 0, that is to say the region is climatically unsuitable, to 1, which means the region is climatically suitable. A 10% training presence threshold was used (Ficetola et al., 2007; Phillips and Dudik, 2008) for the transformation of the modelled probability of occurrence into binary predictions of species presence or absence and then 90% of the data is used in developing the model. Output files of the current and future projections from MaxEnt were converted to raster format using ArcGIS software (ESRI ArcGIS 10). Shifts in the geographical range extent of potentially suitable habitats were calculated using the raster calculator in spatial analyst tools of ArcGIS (O’Donell et al., 2012).
Figure 6A: An example of area under curve (AUC) Jacknife showing different climate variables (bio1-19) used in predicting the potential spread of *Crotalaria agatiflora*, with variables >0.75 considered to have good predictive potential than variables <0.75.
2.3.8 DNA sequencing and isozyme studies

To confirm the morphological identification of *C. agatiflora* and to explore possible genetic variation in the South African material, a DNA barcoding approach (Lahaye et al., 2008) was followed. Leaf material was ground by hand prior to DNA extraction. Whole genomic DNA was extracted using a modified cetyltrimethyl ammonium bromide (CTAB) method as described by Doyle and Doyle (1990). The *trnH-psbA* gene region was amplified using the five chosen populations across the three provinces in South Africa. Polymerase Chain Reaction (PCR) consisted of a thermocycle of an initial denaturation of 95 °C for 5 min; 35 cycles at 94 °C for 30 s, 58 °C for 60 s, elongation at 72 °C for 90 s; and final extension at 72 °C for 10 min. Amplified, double stranded DNA fragments were purified using the QIAquick PCR Purification Kit (Qiagen, USA) and sequenced in both directions using the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction kit (PE Applied Biosystems) and an automated sequencer (ABI PRISM 377XL DNA sequencer, PE
Applied Biosystems). Sequence data were visualized and edited manually using the Bioedit software version 7.0.8 (Hall, 1999) and Seaview (Gouy et al., 2010). This region was chosen as it seemed best suited for lower level taxonomic discrimination (Taberlet et al., 1991). To identify similar sequences in GenBank, the DNA sequence data was used in a nucleotide–nucleotide blast search (blastn; Altschul et al., 1997).

For the Isozyme studies leaf material of 25 individual plants were collected and analysed from five different populations (three in Mpumalanga and two in KwaZulu-Natal). The distance between the populations was deemed sufficient to measure polymorphisms or genetic differences. The sample was also used to study possible genetic differences within the populations. Collection, tissue preparation, extraction buffers, electrophoresis, staining of gels, interpretation of results, locus nomenclature and statistical analysis follow Van der Bank et al. (1995b). The following buffer systems were used: HC - a continuous buffer (pH = 6.5) system (Stuber et al., 1977); PO - a discontinuous buffer (electrode pH: 8.2; gel pH= 8.7) system (Poulik, 1957); TC - a continuous Tris-Citric-Acid buffer (pH=6.9) system (Whitt, 1970); and TBE - a continuous Tris- Borate-EDTA buffer (pH = 8.6) system (Goncharenko et al., 1992). Population genetic analyses were undertaken using the BIOSYS- 1 computer package (Swofford and Selander, 1989). The method used to determine relationships between the populations consisted of (a) interpreting electromorphs on gels. These are some of the factors that were included in the analyses of C. agatiflora: (a) Mendelian genetics, (b) computing allelic frequencies at various loci, and (c) converting allelic frequencies into a measure of genetic distance (D) among populations, using Nei's (1978) method.

2.3.9 Weed risk assessment modeling

The potential invasiveness of C. agatiflora in South Africa was tested by means of the Australian weed risk assessment protocol of Pheloung et al. (1999). An assessment of species’ invasiveness is considered after answering questions about the environment, climate suitability and biological factors (Pheloung et al., 1999). This method has been shown to produce accurate results for different geographical regions even though it was initially used for evaluating species introduced to Australia (Gordon et al., 2008; 2010). Some guidelines as published in Gordon et al.
(2010) were followed for applying the Australian weed risk protocol to regions of the world outside of Australia. In answering questions pertaining to climate in the protocol; question 2.01 —Is the species suited to South African climates? The predictions of Richardson and Thullier (2007) of the global distribution of climates similar to those of South Africa were also used. According to Pheloung et al. (1999), a minimum of 10 questions have to be answered for each species with two in the biogeography section, two in the undesirable traits subsection and six of the remaining questions for biology/ecology. The scores are calculated using questions relevant to open agricultural and natural (environmental) habitats following Pheloung et al. (1999). Standard thresholds of invasive risk were established and species with an overall score of 6 or more were rejected because these are high invasive risk species according to the model. Species with a score of 1 to 6 meant that the species requires further evaluation, and a species with score of 0 or less are accepted as low invasive risk species (Pheloung et al., 1999).

2.3.10 Cost calculations for *Crotalaria agatiflora* management

Most of the work was previously done by the Working for Water contractors and the SANBI-ISP group. This is still an on-going project hoping to eradicate this species in South Africa. Different populations of *C. agatiflora* were visited across the Gauteng, North West and Mpumalanga Provinces. The work was completed by the contractors sourced by the Invasive Species Programme of SANBI: cut–stump and foliar spray *C. agatiflora* plants found in the area using herbicide issued by the South African National Biodiversity Institute (SANBI). All the necessary equipment was supplied to undertake the work including the safe-keeping of issued chemicals. Familiarity with the Working for Water policy documents was also ensured on the use of herbicides.
Chapter 3: Results and Discussion

3.1 Identity of the species

Field observations of several populations confirmed that only one subspecies have been introduced into South Africa, namely *C. agatiflora* subsp. *agatiflora*. The subsp. *imperialis* usually has the calyx and upper surfaces of the leaves pubescent to tomentose. The size of the bracts and bracteoles overlap and are not of diagnostic value to distinguish between these two subspecies (Polhill, 1982). According to Polhill (1982), the subsp. *agatiflora* rarely exceeds 2 m in height, while subsp. *imperialis* may be up to 5 m tall. In South Africa, subsp. *agatiflora* grows to a height of up to 4.5 m, perhaps responding to ideal growing conditions. The populations studied were morphologically uniform (invariable) and no differences were observed except for the height of the plants (which are almost certainly phenotypical responses the local habitat, especially soil conditions). The morphological uniformity was confirmed by the DNA study (see section 4.6).

3.2 Population dynamics

The graph below (Fig. 7) seems to show different patterns of growth and expansion in the five categories and this is clearly due to the different sizes of populations and changes each population goes through. For example, the Lowveld population is one of the largest populations in this study (Table 1) but the plants are hardly ever exposed to fire except for controlled fires a few years back and that could be why the number of mature multi-stemmed plants is below 10, old multi-stemmed plants are just above 20 and seedlings started germinating. The Colbyn valley wetland seems to have more seedlings which make sense with the regular burning of the area by the City of Tshwane and the second biggest population in terms of area coverage after Lowveld population. The relatively smaller populations like Faerie Glen, Pinehaven and Howick seem to produce a small number of seedlings and plants generally.
Seeds in the Lowveld population are spread by means of the Crocodile River canal that runs parallel to the population and whenever there is flooding seeds in the water come to rest on the soil where they might get a chance to germinate should there be any disturbance. From observations, fire seems to be an excellent agent for the establishment of this species as populations seem to grow denser after fire exposure, probably due to the breaking of seed coat dormancy. This influences most of the plants under the canopy of this species that are sheltered from absorbing sunlight and limiting the process of photosynthesis in other plants in the community. Seedlings emerge frequently after fires and these fires are mostly human-induced.

Because there was clear evidence of fire in the five studied populations, individual plants in a population were divided into five categories as seen in Fig. 7 above and also referred to in the methodology (section 3.1). Introduction, growth and expansion of a population are affected by many different factors. According to Crawley (1986) and Sakai et al. (2001), recent biological invasions seem to depend on specific variables during invasion, of which Crotalaria agatiflora is not exempt. Invasion of a species follows after a successful introduction and all this takes place in a series of stages that are necessary for a successful process of invasion. These stages include: (1) introducing a species into a new environment, (2) successful colonization.
and establishment of a species and (3) later follows dispersal and secondary spread into other habitats. In addition, genetic changes may also occur on species during these processes and lead to a successful process of invasion (Sakai et al., 2001). Some *Crotalaria agatiflora* populations (e.g. Lowveld and Colbyn) are dense and healthy ranging from seedlings to old mature plants. Others (e.g. Faerie Glen) appear to be still in the lag phase. This seems to suggest a successful invasion process as explained in Sakai et al. (2001).

Table 1: Approximate sizes of *Crotalaria agatiflora* populations in meters (width and breadth) used in this study across three provinces in South Africa.

<table>
<thead>
<tr>
<th>Population</th>
<th>Province</th>
<th>Size of population (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colbyn</td>
<td>Gauteng</td>
<td>21 x 15</td>
</tr>
<tr>
<td>Faerie Glen</td>
<td>Gauteng</td>
<td>17 x 14</td>
</tr>
<tr>
<td>Pinehaven</td>
<td>Gauteng</td>
<td>13 x 8.3</td>
</tr>
<tr>
<td>Lowveld</td>
<td>Mpumalanga</td>
<td>50 x 30</td>
</tr>
<tr>
<td>Howick</td>
<td>KwaZulu-Natal</td>
<td>13 x 9.5</td>
</tr>
</tbody>
</table>

According to Baker (1965) good colonists are generally species that are able to self-fertilize so that isolated individuals can reproduce. In addition, species that are able to reproduce using both vegetative reproduction and seeds are also successful colonists (Sakai et al., 2001). Once a species has successfully colonised and established in a habitat, it may spread over long distances with either the aid of humans or naturally, and also disperse over short distances showing minor to moderate side expansion of the established populations (Davis and Thompson, 2000). Successful dispersal is completed by the ability of a species to adapt to its new environment and this leads to continued establishment of populations. Once established, the causes of spread of a population are known to be consistent (Sakai et al., 2001). Well-known agents of dispersal such as wind, water and birds are involved in the spread of most populations (Schiffman, 1997). *Crotalaria agatiflora* has proven to be somewhat different in the method of dispersal and spread to advance its populations to colonise new habitats. It has been observed in this study that its dispersal appears to be mostly mediated by human activities. These include
disturbance and spread of seeds along roadsides (probably by the transportation and movement of soil) and horticulture (e.g. the dumping of seed-bearing plant material). In other cases, however, the dispersal is by natural means (e.g. fruits and seeds of plants on river banks are dispersed by water). According to Goodell et al. (2000) understanding the biology, especially the dispersal mechanisms of potential invaders, is important for the development of managerial measures to prevent the spread of these species which is often easier than controlling them after establishment.

As part of the study, questions were posed to local people to find out more about the age and history of the populations, to try and estimate the years from which they started seeing the presence of the species in that particular area. This was meant to assist in determining the age of the populations and to see if they are still in the lag phase or the possibility of this species not being a threat if disturbance is controlled. Residents near the Faerie Glen Nature Reserve who have been jogging and hiking there for almost 21 years claim that the plant was well known to them in that area, and that the population has not increased in size. One of the residents of Faerie Glen, Dr. Helmut Zimmermann (pers. comm.) claims that lack of dispersal agents or the probability of a lag phase might be the reason for the slow advancement of this species. A population visited in Mpumalanga seemed to be inhabiting an empty piece of land that looked like an old garden area. According to residents of the area, the population apparently has been established more than 30 years ago. They claimed that the population size has only increased slightly over the years. These observations indicate that *C. agatiflora* is very persistent once established but that it is slow to disperse and to form new populations.

3.3 Soil analysis

It is clear from studying the map (Fig. 8) that *C. agatiflora* does not prefer any particular soil type but that the known populations occur on many different soil types. According to Fey (2010) there are 73 soil forms in the classification system of soil in South Africa and these can be placed into 14 groups. These can be determined using an eliminative key based on the presence or absence of some diagnostic characters or horizons. The results from my analysis of the soil collected at different
populations (Tables 2 and 3) showed consistency with the data from the ARC-ISCW (Fig. 8). Populations are able to inhabit different soil types (Fig. 8). The data on the soil particle size (Table 2) of different soils from different populations have no similarities or any links that might lead one to believe that *C. agatiflora* has a preference for any particular type of soil or soil texture. It is noteworthy, however, that the soils are generally quite sandy (i.e., with more than 50% sand).

Figure 8: Map of soil types in South Africa as presented by the Agricultural Research Council-Institute for Soil, Climate and Water (ARC-ISCW, 2004). The dots on the map represent *Crotalaria agatiflora* populations that have been recorded in the past. List is of all the coordinates is available at: http://data.gbif.org/species/7067713/. Accessed: 18 March 2013.
Table 2: Results of analyses of soil collected from five different populations in South Africa where *Crotalaria agatiflora* is encroaching natural habitat. The analyses were done by the Agricultural Research Council’s Institute for Soil, Climate and Water.

<table>
<thead>
<tr>
<th>SITE</th>
<th>Org. C</th>
<th>N-NH4</th>
<th>P</th>
<th>K</th>
<th>Na</th>
<th>Ca</th>
<th>Mg</th>
<th>pH(H₂O)</th>
<th>Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>mg/kg</td>
<td>mg/kg</td>
<td>mg/kg</td>
<td>mg/kg</td>
<td>mg/kg</td>
<td>mg/kg</td>
<td>*</td>
<td>ohms</td>
</tr>
<tr>
<td>FAERIE GLEN</td>
<td>4.44</td>
<td>4.16</td>
<td>4.57</td>
<td>136.512</td>
<td>1.797</td>
<td>2721.506</td>
<td>749.547</td>
<td>5.72</td>
<td>680</td>
</tr>
<tr>
<td>FAERIE GLEN</td>
<td>4.67</td>
<td>3.60</td>
<td>2.04</td>
<td>176.984</td>
<td>1.36</td>
<td>2826.689</td>
<td>829.233</td>
<td>6.16</td>
<td>1170</td>
</tr>
<tr>
<td>HOWICK</td>
<td>2.85</td>
<td>4.44</td>
<td>3.52</td>
<td>256.068</td>
<td>5.695</td>
<td>1707.766</td>
<td>470.439</td>
<td>6.68</td>
<td>980</td>
</tr>
<tr>
<td>HOWICK</td>
<td>2.98</td>
<td>7.01</td>
<td>3.12</td>
<td>325.900</td>
<td>2.578</td>
<td>2061.826</td>
<td>507.015</td>
<td>6.97</td>
<td>650</td>
</tr>
<tr>
<td>LOWVELD</td>
<td>10.54</td>
<td>10.51</td>
<td>22.47</td>
<td>384.301</td>
<td>9.855</td>
<td>3243.440</td>
<td>882.076</td>
<td>6.26</td>
<td>530</td>
</tr>
<tr>
<td>LOWVELD</td>
<td>8.28</td>
<td>13.56</td>
<td>8.87</td>
<td>475.913</td>
<td>10.173</td>
<td>3158.772</td>
<td>858.861</td>
<td>6.34</td>
<td>470</td>
</tr>
<tr>
<td>PINEHAVEN</td>
<td>3.45</td>
<td>7.88</td>
<td>9.96</td>
<td>449.064</td>
<td>11.514</td>
<td>2381.040</td>
<td>651.168</td>
<td>6.98</td>
<td>530</td>
</tr>
<tr>
<td></td>
<td>2.06</td>
<td>2.33</td>
<td>8.34</td>
<td>168.562</td>
<td>5.485</td>
<td>1501.046</td>
<td>238.855</td>
<td>7.39</td>
<td>1340</td>
</tr>
<tr>
<td>--------</td>
<td>------</td>
<td>------</td>
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<td>-------</td>
<td>-----------</td>
<td>---------</td>
<td>------</td>
<td>-------</td>
</tr>
<tr>
<td>PINEHAVEN</td>
<td>2.26</td>
<td>2.53</td>
<td>3.28</td>
<td>239.814</td>
<td>3.821</td>
<td>2029.943</td>
<td>513.168</td>
<td>6.94</td>
<td>970</td>
</tr>
<tr>
<td>COLBYN</td>
<td>3.58</td>
<td>3.46</td>
<td>10.53</td>
<td>318.742</td>
<td>21.342</td>
<td>4152.446</td>
<td>756.433</td>
<td>7.44</td>
<td>290</td>
</tr>
<tr>
<td>COLBYN</td>
<td>4.23</td>
<td>5.25</td>
<td>53.61</td>
<td>813.237</td>
<td>20.811</td>
<td>4081.519</td>
<td>771.358</td>
<td>7.24</td>
<td>270</td>
</tr>
<tr>
<td>COLBYN</td>
<td>4.64</td>
<td>4.62</td>
<td>5.76</td>
<td>770.740</td>
<td>14.332</td>
<td>3909.082</td>
<td>783.255</td>
<td>7.46</td>
<td>280</td>
</tr>
</tbody>
</table>
Table 3: Particle size distribution of soil collected from different populations of *Crotalaria agatiflora* in South Africa.

<table>
<thead>
<tr>
<th>SITE</th>
<th>SAND (%)</th>
<th>SILT (%)</th>
<th>CLAY (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2–0.05 mm</td>
<td>0.005–0.002 mm</td>
<td>&lt;0.002 mm</td>
</tr>
<tr>
<td>FAERIE GLEN</td>
<td>52.0</td>
<td>22.0</td>
<td>26.0</td>
</tr>
<tr>
<td>FAERIE GLEN</td>
<td>54.0</td>
<td>22.0</td>
<td>24.0</td>
</tr>
<tr>
<td>FAERIE GLEN</td>
<td>52.0</td>
<td>22.0</td>
<td>26.0</td>
</tr>
<tr>
<td>HOWICK</td>
<td>56.0</td>
<td>16.0</td>
<td>26.0</td>
</tr>
<tr>
<td>HOWICK</td>
<td>58.0</td>
<td>16.0</td>
<td>22.0</td>
</tr>
<tr>
<td>HOWICK</td>
<td>62.0</td>
<td>16.0</td>
<td>22.0</td>
</tr>
<tr>
<td>LOWVELD</td>
<td>58.0</td>
<td>18.0</td>
<td>24.0</td>
</tr>
<tr>
<td>LOWVELD</td>
<td>56.0</td>
<td>18.0</td>
<td>26.0</td>
</tr>
<tr>
<td>LOWVELD</td>
<td>62.0</td>
<td>16.0</td>
<td>22.0</td>
</tr>
<tr>
<td>PINEHAVEN</td>
<td>58.0</td>
<td>18.0</td>
<td>24.0</td>
</tr>
<tr>
<td>PINEHAVEN</td>
<td>78.0</td>
<td>10.0</td>
<td>12.0</td>
</tr>
<tr>
<td>PINEHAVEN</td>
<td>50.0</td>
<td>18.0</td>
<td>32.0</td>
</tr>
<tr>
<td>COLBYN</td>
<td>68.0</td>
<td>12.0</td>
<td>20.0</td>
</tr>
<tr>
<td>COLBYN</td>
<td>76.0</td>
<td>10.0</td>
<td>14.0</td>
</tr>
<tr>
<td>COLBYN</td>
<td>62.0</td>
<td>14.0</td>
<td>24.0</td>
</tr>
</tbody>
</table>
Soil contains varying concentrations of ions/nutrients that determine plants health. Twenty nutrients have been generally known or identified as essential for plants to survive. Of these, nitrogen, phosphorus, potassium, calcium, magnesium and sulphur are amongst the macronutrients required in large quantities. Table 2 indicates how the soil samples differ in soil nutrients. The Colbyn and Lowveld populations display the most fertile soils. This agrees with the data in Table 1, which show that the Colbyn and Lowveld population are much larger than the others, with numerous vigorous and healthy individuals. However, this experiment seemed to be futile in showing any patterns of soil preference for *C. agatiflora* – there seem to be no relation to soil type or nutritional value. No major conclusion came out of this experiment that seemed to be useful in discerning the pattern of this species’ population growth and spread. It seems that soil parameters can be eliminated as a determining factor for the spread of this species.

3.4 Soil-stored seed bank

3.4.1 One transect through five populations: Soil samples along transect to determine soil-stored seed banks, lateral seed spread and viability

Results for the transect method showed that *C. agatiflora* seeds do not spread far from the initial population as presented in the results, Fig. 9. From the chart in Fig. 9 it can be observed that seeds are mostly found under the canopy of the population and the more one moves towards the outside the smaller the number of seeds found and ultimately no seeds were found. No seeds were found for any of the five populations 2 m outside of the population canopy except for one population where seeds were found 4 m away from the population (Colbyn). The distance between the holes dug were 2 m apart from each other, in order to estimate the last soil sample (i.e. furthest away from the population perimeter) that still contained seeds. The spread of seeds in this pattern is not an unusual phenomenon, but it becomes very important if one wants to determine how far the seeds can spread outside of the canopy of the plant. The sizes of populations (Table 1) and the number of seeds collected from the soil (Fig. 9) seemed to also be logically correlated: the smaller the population, the smaller the number of seeds that were found in the soil samples.
It was suggested by earlier studies that within the Fabaceae initial seed release from the parent maybe passive, ballistic or explosive (Berg, 1975; Auld, 1996). In Auld (1996) it was reported that some seed may be released a small distance from the plant, as seem to apply to *C. agatiflora*. This species’ pods twist spirally upon dehiscence where primary seed dispersal is short (generally 0–2 m). The direction east to west was chosen for standardisation of the experiment across the different populations. The 25 cm² x 10 cm deep holes from the easterly to westerly direction were dug to determine the spread of the seeds within and outside the canopy of the population of *C. agatiflora* to be able to determine the distances these seeds are capable of spreading. In the process of doing this, possibilities of secondary parties as agents of dispersal were considered, especially after realising that no seeds were found in the fifth population (Faerie Glen). Populations were observed for several months to see if there were any insects, birds or animals that might feed on the seeds or that might carry the seeds to a new habitat. Dispersal of legume seeds may be due to several secondary dispersal mechanisms. The most widespread and best documented is by ants (Auld, 1996). Mammals have also been recorded to be dispersal agents of some legume species like *Desmodium* DC. (McIntyre et al., 1995). Birds are likely to disperse seeds over long distances while ants do so only over short distances. No animals or birds were observed that seemed to feed on or spread the seeds of *C. agatiflora* except Bruchiid beetles, which had normally drilled small holes on the pods to eat the seed. The damage done to these pods and the seeds on the inside are very minimal with an insignificant reduction in production with no visible influence on the spread of this invasive species.
Figure 9: Distribution of seeds in four different populations of *C. agatiflora* across three provinces in South Africa using a transect. The number of seeds collected per hole (6250 cm³) is plotted against the position on the tape measure/transect (m) with 0 as the middle of the population.

Figure 9 shows that Colbyn an the Lowveld populations produce a large number of seed. It remains inexplicable why the Faerie Glen population contained no seeds even after a similar follow-up experiment was done to determine if there are in fact no seeds in the soil. Therefore no chart exists for the Faerie Glen population to show any patterns because of this result (Fig. 9). For other populations, it came as no surprise to observe the pattern of seed spread within the *C. agatiflora* populations (i.e., more seeds in the central part of the population than on the perimeter). By observation, most of the populations seem to invade and persists as one small population until seeds are dispersed to one or more other localities. Dispersal seem to be mostly aided by human interference, as is evidenced by the presence of the species growing around garbage dumps and along roadsides. Also, *C. agatiflora* has
been observed in some gardens around Pretoria and Johannesburg. Routine roadside clearing by the Department of Public Works seem to be the cause for seeds of this species spreading to new localities. Disturbance of populations of this species may create smaller subpopulations around or near the initial point of investation. Hence, *C. agatiflora* populations occur as relatively small but persistent patches. Soil disturbance also transpired to be an important factor in the founding of new populations. My observations showed that soil (which contain seeds) transferred from one place to another results in the emergence of new populations. An area once devoid of plants of *C. agatiflora* is encroached upon because of the contaminated soil or rubble. Fig. 10 shows an example of such human-aided dispersal. *Crotalaria agatiflora* had never been in this area before but the seeds of this species must have came in with the rubble that came from the construction of the student residences of the University of Johannesburg. It seems as though humans play an important role in the growth and expansion of this species in South Africa. People enjoy having it as a garden plant and thus the plant escapes to natural habitats or in the same manner as indicated in Fig. 10. There are also natural ways of dispersal for this species, especially for those invading river edges, watercourses and wetlands but this seems to contribute to a lesser degree than human-mediated dispersal or disturbance.

Figure 10: A new *Crotalaria agatiflora* population in Auckland Park, Johannesburg. The seeds were brought in with rubble (seen as heaps of sand) from another region probably invaded by this species during construction and renovations of student residences. Photos: Thabang Phago.
3.4.2 Three plants in each of four different populations (quadrant method); relationship between pods produced and seed in soil-stored seed bank

The use of the quadrant method for collection of soil samples for the determination of the soil-stored seed bank was mainly to determine the relation between the number of seeds that fall to the ground with the number of seeds produced in the pods and the number of flowers that was initially counted on the racemes. Three mature individuals per population were chosen for this method. Soil was collected on four sides of a mature individual in diagonal lines under the canopy of that particular individual. The quadrant was as shown in Fig. 4C and this was done four times (north, east, south and west) around the same individual, i.e. 12 quadrants of $2 \text{ m}^2 \times 10 \text{ cm}$ for three mature individuals in each population. Seeds collected from this experiment were also used in the germination and seed viability tests as presented later.

Table 3 shows a similar pattern across the three populations. Flowers per raceme were higher than pods per raceme (Table 3) as some flowers died or were not pollinated. The number of seeds per pod (Table 4) are also presented in this section. Firstly, for all three populations the flowers had the highest count, followed by the number of racemes and then the number of pods produced. There seems to be a direct relationship between the number of racemes, flowers and pods of the different populations, i.e. the more flowers counted, the higher the number of pods and seeds produced. This means that the chances of seed production reaching its optimum decreases with every flower on the raceme that does not set fruit. Possible reasons for flowers not developing maximally may include cold weather or frost and lack of pollinators. A statistical analysis of this table was not done but raw data is presented in terms of Tables 3 and 4 detailing the process of counting from raceme level to the production of seeds. In the Colbyn (and Lowveld) populations there seemed to be a better presence of pollinators than Faerie Glen and Pinehaven. Also, from the soil analyses results, the Lowveld and Colbyn populations soil seem to be more fertile than the other two populations which could be the explanation for the high seed yield. The low number of flowers and seeds produced by the Faerie Glen population remains unexplained.
Table 3: The total number of racemes, flowers and pods produced in three mature individuals per population of *Crotalaria agatiflora*. This was done for three populations in Gauteng Province.

<table>
<thead>
<tr>
<th>Number of mature plants</th>
<th>Colbyn population</th>
<th>Pinehaven population</th>
<th>Faerie Glen population</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Racemes</td>
<td>Flowers</td>
<td>Pods</td>
</tr>
<tr>
<td>1</td>
<td>80</td>
<td>442</td>
<td>225</td>
</tr>
<tr>
<td>2</td>
<td>74</td>
<td>481</td>
<td>339</td>
</tr>
<tr>
<td>3</td>
<td>68</td>
<td>228</td>
<td>98</td>
</tr>
</tbody>
</table>

Table 4: The total number of seeds counted for all three populations of *Crotalaria agatiflora* in Gauteng Province.

<table>
<thead>
<tr>
<th>Population</th>
<th>North Quadrant</th>
<th>East Quadrant</th>
<th>South Quadrant</th>
<th>West Quadrant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colbyn 1</td>
<td>582</td>
<td>1588</td>
<td>734</td>
<td>573</td>
</tr>
<tr>
<td>Colbyn 2</td>
<td>1789</td>
<td>1604</td>
<td>389</td>
<td>239</td>
</tr>
<tr>
<td>Colbyn 3</td>
<td>1128</td>
<td>1669</td>
<td>746</td>
<td>124</td>
</tr>
<tr>
<td>Pinehaven 1</td>
<td>766</td>
<td>383</td>
<td>265</td>
<td>396</td>
</tr>
<tr>
<td>Pinehaven 2</td>
<td>761</td>
<td>418</td>
<td>236</td>
<td>219</td>
</tr>
<tr>
<td>Pinehaven 3</td>
<td>363</td>
<td>115</td>
<td>510</td>
<td>106</td>
</tr>
<tr>
<td>Faerie Glen 1</td>
<td>2</td>
<td>-</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Faerie Glen 2</td>
<td>-</td>
<td>5</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Faerie Glen 3</td>
<td>-</td>
<td>1</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>
Table 5: The actual total number of seeds found in the soil in comparison with the amount of seed that was expected to have been found in the soil estimated from the number of seeds produced from the pods.

<table>
<thead>
<tr>
<th>Mature plants per population</th>
<th>Expected total number of seeds per individual</th>
<th>Actual total number of seeds per individual</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colbyn 1</td>
<td>11700</td>
<td>3477</td>
</tr>
<tr>
<td>Colbyn 2</td>
<td>17628</td>
<td>4021</td>
</tr>
<tr>
<td>Colbyn 3</td>
<td>5096</td>
<td>3667</td>
</tr>
<tr>
<td>Pinehaven 1</td>
<td>6708</td>
<td>1810</td>
</tr>
<tr>
<td>Pinehaven 2</td>
<td>4732</td>
<td>1634</td>
</tr>
<tr>
<td>Pinehaven 3</td>
<td>6188</td>
<td>1094</td>
</tr>
<tr>
<td>Faerie Glen 1</td>
<td>104</td>
<td>3</td>
</tr>
<tr>
<td>Faerie Glen 2</td>
<td>312</td>
<td>9</td>
</tr>
<tr>
<td>Faerie Glen 3</td>
<td>676</td>
<td>7</td>
</tr>
</tbody>
</table>

Soil samples were sieved after the soil was collected from different populations and the seed counted. The number of seeds that reach the soil is the result of opening of healthy pods where the number of pods depends on the number of flowers pollinated and that in turn depends on the number of flowers on a flower stalk or raceme. This is a long chain of natural events that allow growth and expansion in plant populations (De Toledo et al., 2003). Pollination plays an important role in determining the number of pods produced per plant. This species is pollinated by birds and the flowers succumb to different conditions, both natural and unnatural, whether by freezing temperatures, frost or lack of pollinators in a particular habitat and this affects the number of seeds produced and thus also population growth (De Toledo et al., 2003). The actual total number of seeds collected from the soil-stored seed bank was compared to the expected total number of seeds when the number of pods produced from flowers (Tables 3-5) was calculated. From these data is was possible to estimate the number of seeds that should reach the ground for germination. The Faerie Glen population produced a very low number of seeds. A more detailed discussion follows under the reproductive biology section below.
Different aspects of reproduction or the advancement of invasive plants have for a long time been of concern especially seed number, seed size and reproductive potential (Schaal, 1980). *Crotalaria agatiflora* produces a large number of small-sized seeds (Fig. 11, Table 5) but not all the seeds reach the soil or get a chance to establish. Seed size has been considered a strong selection character, as small seeds contain less endosperm than large seeds and this leads to a decreased number of seedling survivors and a low competitive ability (Harper, 1977). Could this be the reason why *C. agatiflora* has not reached its optimum population growth that would be expected when one considers the large number of seeds produced per mature individual plant? Could it be that the less endosperm contained in the small seeds continuously leads to less chances of seedling surviving and competing? Of course in the same light, larger seeds may also be selected against since they may be less freely available (Baker, 1972) or may be subjected to higher levels of
predation (Smith, 1974). On the other hand, larger numbers of small seeds may produce dense stands of seedlings that undergo greater competition (Black, 1957). However, size, weight and number of seeds are not the only limiting factors in invasion biology. Other mechanisms include pollination, use by predators and human mediation or disturbance.

3.5 Reproductive biology

This section of the results deals with the field observations on reproductive potential of *Crotalaria agatiflora* which are mainly general observations (Fig. 12 and 13). Observations are discussed in reference to literature. None of the observations have been quantified but a detailed description is given. Reproduction is loosely known as the production of new organisms whether plants or animals and can be achieved by either sexual or asexual means. It is important in the advancement and growth of populations (Holsinger, 2000). *Crotalaria agatiflora* reproduces sexually by using birds to transmit pollen from flower to flower. Therefore it is likely that population advancement and growth is through the spread of seed and not vegetative reproduction. There are many factors that need to be taken into consideration when determining the growth rate or advancement of a population. Insects like bruchid beetles that seem to feed on the seeds of *Crotalaria* species, the presence or absence of pollinators and the availability of animals that might transport the species to new habitats are factors that need to be considered when determining the spread of the population (Sakai et al., 2001). An example can be observed below in Fig. 12 where holes in the pods were created by bruchid beetles. In some of these pods, fungal infections prevented the formation of mature seeds, thus affecting reproduction. Bruchids or weevils are known to be closely associated with leguminous species (Sales et al., 2000; Fox et al., 2012) and this was also observed for *C. agatiflora* in the wild. Although seed predation is common amongst many species of plants, in *C. agatiflora* weevil predation does not seem to have a large impact in significantly reducing the production of seeds because these predators are only observable in some *C. agatiflora* populations, which also give an indication of the seriousness of the predation. Also, a collection of many healthy seeds from the
soil-stored seed bank of different populations further indicated the minimal effect of predation on reproduction.

![Image of Crotalaria agatiflora pods with evidence of weevil boring. Weevils (bruchid beetles) bore through the fruit walls to feed on the seeds. Photos: Thabang Phago.](image)

**Figure 12:** *Crotalaria agatiflora* pods with evidence of weevil boring. Weevils (bruchid beetles) bore through the fruit walls to feed on the seeds. Photos: Thabang Phago.

Some other predators have been observed on *C. agatiflora* plants. These were probably general feeders and were found on the leaves and twigs and not the flowers or fruits. These leaf feeders make a lesser impact on reproduction than the bruchids or weevils feeding on the seeds. Leaf miners were observed on the leaves of some plants of *C. agatiflora* (Fig. 13). These miners eat the leaves of *C. agatiflora* and also seem to have minimal impact on the growth and advancement of the populations of this species. Some of these predators were collected from *C. agatiflora* in this study. However, Spencer (1990a) stated that *Crotalaria* is the only single genus known to be colonized by Agromyzidae, a family of leaf miner flies. In Uganda, *Melanogromyza crotalariana* has been found on stems of *Crotalaria* species and in Kenya an *Ophiomyia* species (*O. crotalariella*) was found to have been forming external mines in young, terminal twigs of *C. agatiflora* (Spencer, 1990a). The presence of the *Ophiomyia* species is revealed by leaf drop and the withering of the twigs that are affected. Another insect species detected on *C. agatiflora* is
*Japanagromyza parvula*. It was found to form blotch mines on plants growing on the grounds of the herbarium of the National Museum of Kenya (Spencer, 1990a).

Figure 13: Examples of predation on *Crotalaria agatiflora*. Leaves are bored and mined by feeding insects. Photos: Thabang Phago.
In reproduction, pollinators play a significant role in transferring pollen of the plant to female parts. This in turn produces fruits and then seeds for the advancement of the life cycle of the plant to continue. In the study of pollination ecology, pollinator exclusion experiments are often used to answer various questions relating to reproductive biology (Thomson et al., 2011). In this study some inflorescences were excluded from pollinators to determine the role of pollinators in successful seed set in this species and to see if self-pollination is possible. This technique is widely used and popular among entomologists and researchers working in biocontrol agents. Pollination bags are usually used for sliding over a shoot, enclosing it by pulling a drawstring to wrap it around the stem (Thomson et al., 2011). The bag lets in air, sunlight and elements necessary for the plant to continue functioning normally. While the bags are successful at keeping out the pollinators, they do result in physical disturbance of the environment, also during time it takes to secure and remove the bags (Thomson et al., 2011).

From the bagged racemes no fruit set was observed; instead all flowers died and fell off the plant. This shows the crucial importance of pollinators in the growth and advancement of *C. agatiflora* population. Some open flowers that were included in the bags as control (Fig. 14) did produce seed in the normal way but all others did not. Without pollination the production of seeds is impossible, thus limiting the spread of *C. agatiflora*. 
Although flowers from the tribe Crotalarieae (of which Crotalaria is a member), are generally adapted to be pollinated by bees (Mitchelmore, 1990; Le Roux and Van Wyk, 2012), bird pollination is also found in several African species either with some modification or occasionally with reduction of the wings and extra strengthening in the sepals, as in Crotalaria rosenii (Pax) Polhill (Yeo, 1993). Crotalaria agatiflora has been observed to be visited by both honeybees and birds. However, honeybees visit the species for nectar but are unable to reach the female parts of the plant that require the transfer of pollen for pollination. Sunbirds, however, are well-suited to successfully transfer the pollen from the male to female parts of the plant and benefit from the nectar produced by the plants. Different sunbirds were observed during pollination studies of C. agatiflora. The species of sunbirds that were photographed include Chalcomitra amethystina (amethyst sunbird, black sunbird), Nectarinia kilimensis (bronze sunbird) and Cyanomitra veroxii (grey sunbird). Although no large bees of the family Xylocopidae were observed during field studies, the presence of sculpturing on the wing petals of the flowers indicates that C. agatiflora is adapted to also be pollinated by large xylocopid bees. It will be interesting to confirm this
assumption in the natural distribution range of the species and to make further observations in South Africa to see if xylocopid bees can pollinate *C. agatiflora*.

The importance of seed viability testing needs no emphasis especially in understanding and managing plants that have persistent seed banks (Sawma and Mohler, 2002). Seed viability and germination are only shown in photographs in the results section and no raw data was collected as the objective was to see whether seeds are viable/germinate or not. Photographs of viable and germinating seeds are shown (Fig. 15, 16 and 17) as evidence. *Crotalaria agatiflora* records in South Africa date back to 1921, with a first record in the Pretoria National Herbarium from a specimen collected in Cape Town (Jaca et al., 2013). This means that the species has persisted and may have been spreading for almost a century in South Africa. It is likely that many seeds have been produced over this time which may survive for many years in the soil. Seed coat dormancy and associated longevity of the seeds is a well-known mechanism in legumes seeds that also seems to be employed by *C. agatiflora* to survive. A seed that is described as viable is one that in itself has the ability to germinate. Viability is the amount or proportion of viable seeds in a particular seed lot or a particular selected area (Sawma and Mohler, 2002). Testing seeds for viability has been used in a wide variety of studies, including restoration, conservation ecology, natural ecosystems and on agricultural weeds, particularly those associated with frequent fires (Ashton et al., 1998; Morgan, 1998; Whittle et al., 1998). Several methods for seed viability testing have been established and most have in previous studies shown to be laborious and have limited the scope of seed bank studies, with each test having its advantages and disadvantages. Tetrazolium testing, also popularly known as TTZ testing, provides a quick way to obtain seed viability data, but can also be laborious and its application to small seeds requires much skill (Sawma and Mohler, 2002). It is common knowledge that seeds play an important role in the survival, growth and advancement of populations. This means that for populations of any species to establish and keep surviving, the plants have to continue producing seeds (unless the species can reproduce by other means e.g. by vegetative reproduction).

Ellis and Roberts (1980) suggested that the temperature and moisture content are mainly responsible for the rate (but not the nature) of the ageing process of seeds of barley and many other species. It seems that seeds of *C. agatiflora* can remain in the
soil for a long time before scarification takes place and this is most likely caused by fire in fire-prone areas (Auld, 1996). Scarification and imbibition are essential steps without which germination is not possible.

In the present study, only seeds that were about three years old or younger were available for determining the viability and longevity of the seeds in the soil. Some other legume seeds are known from other studies to last for longer than 20 years (Auld, 1996). The results of all my experiments showed 100% viability and germination. The tetrazolium method (Peters, 2000) proved useful in determining the viability of the *C. agatiflora* seeds. However more studies are needed to determine the longevity of the viable seeds in the soil over a longer period of time (i.e. 20 years or longer). This method of Peters (2000) was verified by testing other legume seeds for comparison. *Vicia faba* L. seeds from the same family of legumes were used as positive control (Fig. 16) and non-viable *Xanthocercis zambesiaca* Baker seed were used as negative control. Some seeds were not scarified to test for the level of seed coat dormancy. No germination or viability was observed or recorded for seeds that were not scarified. A 100% viability and germination was observed for all the seeds that were scarified and tested for viability and germination. *Crotalaria agatiflora* seeds remain dormant in the soil and await favourable conditions. The heat on the soil produced by fire and possibly also mechanic scarification (e.g. wheels of vehicles and heavy equipment on roadsides) are likely the main causes of scarification and therefore essential elements in the germination and spread of populations of *C. agatiflora*. 
Figure 15: Tetrazolium testing of *Crotalaria agatiflora* seeds. The original pale brown seeds (top row) turn purple red after testing (bottom row), thus showing viability (all seed lots tested were 100% viable). Photos: Thabang Phago.

Figure 16: *Vicia faba* seeds used in the tetrazolium test as positive control. All seeds stained purple red indicating a positive test for viability. Photos: Thabang Phago.
Auld and O’Connell (1991) have shown how dry heat is able to break seed dormancy in legumes, a pattern that is known to be common in legumes found in fire-prone areas throughout the world (Keely, 1981; Jeffrey et al., 1988). Legume seeds within a narrow band of soil depth are generally stimulated by fire to germinate and temperatures of 120˚C or more are deadly except for very short (1 minute) durations. Although other disturbance mechanisms may play a role in certain vegetation communities (like flooding in swamps, or formation of gaps in rainforests), the impact of fire can be seen in all plant communities (Auld, 1996). Species response to fire varies according to distribution and various factors.

Germination experiments were undertaken (Fig. 17) to further verify the tetrazolium testing results. Legumes generally have hard dormant seeds with an impermeable testa that prevents the uptake of water, hence making the seed dormant (Tran and Cavanagh, 1984; Van Staden, et al., 1989) and this is also the case for *C. agatiflora*. The differences in seed dormancy have been attributed to seed size (i.e. high seed dormancy occurs in large-seeded species) and seed testa structure (Morrison et al., 1992). No germination occurred without any scarification of the seed first. 100% germination was observed for all scarified seeds but the seeds tested were all less than three years old (Fig. 18). It will be important to test older seed in order to determine the maximum longevity of the seed when it becomes part of the seed soil bank. Long-lived seeds will result in the need for regular follow-up operations as part of an eradication strategy.

![Figure 17: Germination of *Crotalaria agatiflora* seeds in an incubator. There was no germination without scarification of the seeds first and 100% germination of scarified (imbibed) seeds. Photos: Thabang Phago.](image-url)
3.6 Climatic modelling

Bioclimatic modelling of species distributions has become very important in ecology and conservation for a variety of applications (Graham et al., 2004). Thuiller et al. (2005) reported that predictive models are useful when studying the spread of invasive species. Some of these models are known to assist in determining the impacts of climate change and its effect on invasive species (Thomas et al., 2004) and spatial patterns of species diversity (Graham et al., 2006). According to Phillip and Dudik (2008), presence-only modelling methods only require locality records of the species together with predictor variables. These variables can range from topographic, climatic, edaphic, biogeographic and remotely sensed variables.

In this study, MaxEnt was used as a presence-only method, together with climatic variables, to predict the potential area of invasion by *C. agatiflora*. In a recent broad comparative study of presence-only modelling techniques by Elith et al. (2006), some new methods were shown to have better predictive accuracy than the established methods and one of these methods is MaxEnt. A feature that separates the new techniques from the established ones is the ability to fit more complex models from smaller datasets, using regularization mechanisms to prevent model complexity from increasing over what is supported by the input data (Phillip and Dudik, 2008).

Species distribution modelling in MaxEnt produced a very high accuracy when projected onto the global distribution records, acquired from the Global Biodiversity Information Facility (GBIF). Area under curve (AUC) = 0.997; sensitivity = 0.975; specificity = 0.924 and test data were correctly classified. *Crotalaria agatiflora* invades some parts of South Africa (Fig. 19), and around 50–60% of South Africa is climatically suitable for the growth of this species. Only one province (Northern Cape Province) seems unlikely to be invaded even when conditions are projected into the future (Fig. 19). Collectively all bioclimatic variables contributed to a varying degree but the most contribution came from Bio 12 = Annual precipitation, Bio13 = Precipitation of wettest month, Bio 15 = Precipitation seasonality (coefficient of variation), Bio 16 = Precipitation of wettest quarter and Bio 18 = Precipitation of warmest quarter (Fig. 6A, 6B). Potential distribution as modelled in MaxEnt can be visualised in the Figs 18–20. The potential distribution of *C. agatiflora* follows the
current natural spread of this species as recorded by the Southern African Plant Invaders Atlas (SAPIA) and GBIF. This is also indicative of the sensitivity and accuracy of the model that was used in this study. The spread is mainly in the northern parts of South Africa (Limpopo, Gauteng, Mpumalanga and North West Provinces) inhabiting also the eastern coastline from Mpumalanga right through to KwaZulu-Natal, Eastern Cape and the Western Cape.

Three models were run, one for the current climate scenario (Fig. 18) and two for the future climate scenario (Figs 20, 21). Two scenarios were selected for the future scenario to compare the differences in the potential range between these climate scenarios (CCCMA and CSIRO for 2080). There were no major differences between these two scenarios. The potential range of invasion expanded for both scenarios for the year 2080. However, the CSIRO scenario showed a lesser expansion range (about 0.5 km) than the CCCMA (about 1.2 km) although the overall increased potential range is just less than 1.5 kilometres.

Figure 18: The current distribution of *Crotalaria agatiflora* according to the Southern African Plant Invaders Atlas (SAPIA) on the left and the current potential distribution of invasion by *C. agatiflora* in South Africa (on the right) as predicted by modelling.
Figure 19: The future potential distribution of *Crotalaria agatiflora* projected to the year 2080 for both the CCCMA (left) and CSIRO (right) climate scenarios. Minor differences are evident.

Figure 20: The expected changes in the distribution of *C. agatiflora* in the year 2080 according to the bioclimatic variables (Bio 1-19) used to determine potential range of invasiveness with CCCMA (left) and CSIRO (right).
3.7 DNA sequencing and Isozyme studies

The DNA sequencing yielded no polymorphic regions for invasive populations of *C. agatiflora*. There were no discrepancies between any nucleotides that could raise questions around genetic variation of this species. Sequences of all six populations sampled appear to be the same, as illustrated below by the sequences. The *trnH-psbA* matrix contained about 319 characters. The matrix is available upon request, but shows clearly that there are no polymorphic areas.

Because there appeared to be no polymorphic areas in the DNA sequences, enzyme electrophoresis was used as a second and potentially more sensitive method for determining genetic variation between populations (Soltis and Soltis, 1989). Different allozymes were used to see if there is variation between the populations of *C. agatiflora*. All the isozymes/allozymes that were used to detect variability are indicated in Table 6. None of these isozymes showed any genetic variation as was also the case with DNA sequencing. Fig. 22 shows four photographs of gels that are a clear indication that the carefully selected populations sampled were not at all different. This provides evidence that these populations originated from the same seed source, thus arguing against the idea of multiple introductions. The tree (Fig. 22) and branch lengths (Table 7) further seem to eliminate any doubt that the species was introduced only once and not more than one from different genotypes. All the statistic analyses of the isozyme study are available upon request.

Table 6: Branch lengths and linkages for an unrooted Wagner network after optimization, based in isozyme data of *Crotalaria agatiflora* and *C. capensis*.

<table>
<thead>
<tr>
<th>OTU or HTU</th>
<th>HTU</th>
<th>Branch length</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPUMALANGA</td>
<td>4</td>
<td>.000</td>
</tr>
<tr>
<td>OUTGROUP</td>
<td>4</td>
<td>.424</td>
</tr>
<tr>
<td>KWAZULU-NATAL</td>
<td>4</td>
<td>.000</td>
</tr>
</tbody>
</table>
Figure 21: Wagner tree produced after the variation between the population in Mpumalanga and KwaZulu Natal were compared with the outgroup.

Figure 22: Gel electrophoresis photos of four different isozymes compared to detect possible variation between two populations of *Crotalaria agatiflora* under study (with *C. capensis* as the outgroup). Photos: Thabang Phago.
3.8 Weed risk assessment modelling

*Crotalaria agatiflora* has had some invasion history recorded from around the world and different subspecies of this species have also been documented to occur elsewhere in the world (GBIF, 2013). Table 7 shows the number of questions answered for the purposes of this assessment in the present study. All questions could be answered except three regarding natural hybridisation, shade tolerance and secondary dispersal by animals. From an experiment done in the previous section (Section 4.7) to determine any possibilities of variation between populations of this species and across species in one population, it is clear that no evidence of hybridisation exists at this point thus the question was left unanswered. Also no evidence of secondary propagule dispersal by animals was observed for any of the *C. agatiflora* populations studied for the entire period of the study.

Another part of importance in the Pheloung et al. (1999) model of assessing the risk of a particular emerging weed is the role of climate in the distribution of the species under investigation. *Crotalaria agatiflora*’s potential distribution range was modelled and calculated in km for the year 2080 to answer the questions on part 2 of the assessment. All the details for this study are in the previous section (Section 4.4). All questions were answered confidently with either evidence from literature or observations in the field.

Generally, prevention of the introduction of alien invasive plants is the most cost effective method of limiting the consequences of biological invasions with a high risk of becoming invasive, while also allowing entry of species that are low risk and may possess social and economic worth (Hulme, 2006; Keller et al., 2007). Rejmánek et al. (2005) reported the difficulty of differentiating the characteristics defining high risk or low risk species within invasion biology. It was left to Pheloung et al. (1999) to develop a weed screening system based on answering a number of questions that relate to biogeography, invasion history, traits that are undesirable and the ecology of the species under investigation. This model is known as the Australian weed risk assessment (A-WRA) model. The A-WRA model is widely used and has been consistently tested. It quantifies invasion risk of species and allows one to make decisions to ‘reject’, ‘accept’ or ‘further evaluate’ a species (Gordon et al., 2008). The success of A-WRA has led to its incorporation into national policy in Australia and
New Zealand, and has proven to be very useful economically when applied (Keller et al., 2007). The A-WRA was further developed specifically for the tropical systems of Hawaii and the Pacific Islands (Daehler et al., 2004). The Hawaiian weed risk assessment (H-WRA) model has a strong focus on tropical climate and soil questions with the intention to adjust them geographically (Gordon et al., 2008), as well as an additional screening step to reduce the number of species requiring further evaluation.

Both these models were shown to have several limitations (Daehler et al., 2004; Dawson et al., 2009). Species that are poorly studied are faced with the risk of scoring very low for invasion risk. Another limitation is that the score from the WRA is strongly influenced by whether a species has a strong invasion elsewhere (Caley and Kuhnert, 2006; Weber et al., 2009). However, many invasive weeds may have no history of introduction (Dawson et al., 2009). According to Williams et al. (2001) in New Zealand, 20% of naturalised weeds had never been recorded before as invasive. Successful invasion can depend on factors like propagule pressure (Lockwood et al., 2005) and the time since introduction (Wilson et al., 2007). In a study by Krivanek and Pysek (2006), the H-WRA model provided the best results when compared to the A-WRA and decision tree scheme of Reichard and Hamilton (1997). Overall accuracy of H-WRA was higher than both A-WRA and the Reichard-Hamilton method, although the A-WRA model which was used for this study rejected 100% of invasive species.

An overall total score of 17 was obtained for *C. agatiflora* which indicates the extent of the invasive potential of the species in South Africa. The score partitioning was 6 for biogeography, 7 for undesirable attributes and 4 for biology/ecology (Table 8). This score is very high compared to the score of 6 or less that is required for a plant to be considered as a low risk species. The quality of the data acquired to answer the questions and the number of questions answered shows that the results of the Australian weed risk assessment model are reliable and that *C. agatiflora* can be considered as a high invasive risk species that is suited to South African climates. One can therefore deem the assumption to categorize this species on the basis of its observed of weedy tendencies as a category 1a species to be fully supported. Furthermore, considering the results of this modelling, I can suggest that *C.*
agatiflora can now be moved to category 1b, meaning it needs to be controlled as part of a management plan.
Table 7: Weed risk assessment model questionnaire for *Crotalaria agatiflora*.

<table>
<thead>
<tr>
<th>Weed Risk Assessment System</th>
<th>Outcome:</th>
<th>Score:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. History/ Biogeography</strong></td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>Domestication/cultivation</td>
<td>1.01 Is the species highly domesticated? If answer is 'no' go to 2.01</td>
<td>Y</td>
</tr>
<tr>
<td>1.02 Is species naturalised where grown?</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>1.03 Does the species have weedy races?</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>Climate and Distribution</td>
<td>2.01 Species suited to Australian climates (0-low; 1-intermediate; 2-high)</td>
<td>2</td>
</tr>
<tr>
<td>2.02 Quality of climate match data (0-low; 1-intermediate; 2-high)</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>2.03 Broad climate suitability (environmental versatility)</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>2.04 Native or naturalised in regions with extended dry periods</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>2.05 Does the species have a history of repeated introductions outside its natural range?</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td><strong>B. Biology/Ecology</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Undesirable traits</td>
<td>4.01 Produces spines, thorns or burrs</td>
<td>N</td>
</tr>
<tr>
<td>4.02 Allelopathic</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>4.03 Parasitic</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>4.04 Unpalatable to grazing animals</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>4.05 Toxic to animals</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>4.06 Host for recognised pests and pathogens</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>4.07 Causes allergies or is otherwise toxic to humans</td>
<td>E</td>
<td></td>
</tr>
<tr>
<td>4.08 Creates a fire hazard in natural ecosystems</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>4.09 Is a shade tolerant plant at some stage of its life cycle</td>
<td>E</td>
<td></td>
</tr>
<tr>
<td>4.10 Grows on infertile soils</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>4.11 Climbing or smothering growth habit</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>4.12 Forms dense thickets</td>
<td>E</td>
<td></td>
</tr>
<tr>
<td>Plant type</td>
<td>5.01 Aquatic</td>
<td>N</td>
</tr>
<tr>
<td>5.02 Grass</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>5.03 Nitrogen fixing woody plant</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>5.04 Geophyte</td>
<td>E</td>
<td></td>
</tr>
<tr>
<td>Reproduction</td>
<td>6.01 Evidence of substantial reproductive failure in native habitat</td>
<td>N</td>
</tr>
<tr>
<td>6.02 Produces viable seed</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>6.03 Hybridises naturally</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>6.04 Self-fertilisation</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>6.05 Requires specialist pollinators</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>6.06 Reproduction by vegetative propagation</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>6.07 Minimum generative time (years)</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>Dispersal mechanisms</td>
<td>7.01 Propagules likely to be dispersed unintentionally</td>
<td>Y</td>
</tr>
<tr>
<td>7.02 Propagules dispersed intentionally by people</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>7.03 Propagules likely to disperse as a produce contaminant</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>7.04 Propagules adapted to wind dispersal</td>
<td>E</td>
<td></td>
</tr>
<tr>
<td>7.05 Propagules buoyant</td>
<td>E</td>
<td></td>
</tr>
<tr>
<td>7.06 Propagules bird dispersed</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>7.07 Propagules dispersed by other animals (externally)</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>7.08 Propagules dispersed by other animals (internally)</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>Persistence attributes</td>
<td>8.01 Prolific seed production</td>
<td>Y</td>
</tr>
<tr>
<td>8.02 Evidence that a persistent propagule bank is formed (&gt;1 yr)</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>8.03 Well controlled by herbicides</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>8.04 Tolerates or benefits from mutilation, cultivation or fire</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>8.05 Effective natural enemies present in Australia</td>
<td>N</td>
<td></td>
</tr>
</tbody>
</table>

Outcome: Score: 17

Statistical summary of scoring

<table>
<thead>
<tr>
<th>Biogeography</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undesirable attributes</td>
<td>7</td>
</tr>
<tr>
<td>Biology/ecology</td>
<td>4</td>
</tr>
<tr>
<td>Questions answered: Biogeography</td>
<td>8</td>
</tr>
<tr>
<td>Undesirable attributes</td>
<td>11</td>
</tr>
<tr>
<td>Biology/ecology</td>
<td>22</td>
</tr>
<tr>
<td>Total</td>
<td>41</td>
</tr>
<tr>
<td>Agricultural</td>
<td>9</td>
</tr>
<tr>
<td>Environmental</td>
<td>17</td>
</tr>
</tbody>
</table>

A = agricultural, E = environmental, C = combined
3.9 Management options for *Crotalaria agatiflora*

The introduction of *C. agatiflora* in South Africa has led to its spreading to new habitats since its initial introduction which is believed to have been in Cape Town (Jaca et al., 2013). Currently, more than 35 populations have been recorded and more are continuously being discovered. This has led to the categorising of the species as per the National Environmental Management: Biodiversity Act (NEM:BA) as a category 1a species which means that compulsory control is needed and that trading is prohibited by law. It is important that all measures are taken to ensure that further spread is limited, so that experimental studies and control measures can be put in place.

The South African National Biodiversity Institute-Invasive Species Programme (SANBI-ISP) embarked on the control of *C. agatiflora* parallel to ongoing experimental studies. This programme aimed to control the species by mechanical and chemical means using herbicides and machinery. The herbicide used was imazapyr (chopper) and was issued by the SANBI-ISP as the suitable herbicide for the control of *C. agatiflora*.

In this study, three populations of different sizes were selected for experimenting with mechanical control, including two populations from Pretoria (Woodlands and Voortrekkerhoogte) and another from the west of Johannesburg (Pinehaven in Krugersdorp).

All the populations were subjected to similar control methods, even though the populations were not of the same size. The population at Voortrekkerhoogte was the largest in terms of square meterage but the number of individuals in the population was very small. The Woodlands population was the second largest population but possessed more mature individuals and seedlings than the other two populations studied. All the numbers of the individuals and seedlings are listed in Table 8.

All visible individuals were cut just above ground level and treated with herbicide (imazapyr). Seedlings were uprooted.
Table 8: Treatment of three *Crotalaria agatiflora* populations in Gauteng Province.

<table>
<thead>
<tr>
<th>Population</th>
<th>Woodlands</th>
<th>Voortrekkerhoogte</th>
<th>Pinehaven</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mature individuals</td>
<td>~114</td>
<td>~36</td>
<td>~22</td>
</tr>
<tr>
<td>treated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seedlings uprooted</td>
<td>~186</td>
<td>~85</td>
<td>~15</td>
</tr>
<tr>
<td>No. of labourers</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Time</td>
<td>3hrs 20 min</td>
<td>2 hrs</td>
<td>1hr 30 min</td>
</tr>
</tbody>
</table>

There was a varying degree of success when using the herbicide of choice. Imazapyr is a broad-spectrum herbicide that controls many plants and broad-leaf weeds when applied pre-emergence and post-emergence (Kent et al., 1991). Because of its long known history it was chosen as herbicide for controlling *C. agatiflora*. Some plants re-sprouted after having been treated and others died after treatment (Table 9).

Fabaceae species display one of two fire response strategies, namely seeding (plants are killed by fire but persist as seeds in a seed bank) and sprouting (plants re-sprout via root suckers or a woody basal lignotuber (Auld, 1996). It was clear from my field studies that *C. agatiflora* is a sprouting species.

Out of 114 plants that were treated and 186 seedlings uprooted (Table 8) at Woodlands, 46 mature individuals re-sprouted (Table 9). After one month of monitoring and follow-up, 190 new seedlings were counted (Table 9). The numbers of individuals before treatment and after treatment for the other two populations (Voortrekkerhoogte and Pinehaven) are also shown in Table 9. Individuals that appeared to have been missed during treatments are also recorded in Table 9 after one month follow-up. Imazapyr seems successful but requires regular monitoring to eliminate re-sproutings and plants that appear to have been missed during initial control attempts. Seedlings that emerge from seed in the soil-stored seed bank after the initial control activity also need to be removed.
Table 9: The number of plants counted after monitoring and following up on the populations of *Crotalaria agatiflora* subjected to mechanical and chemical control as one combined treatment.

<table>
<thead>
<tr>
<th>Population</th>
<th>No. of seedlings</th>
<th>Resprouting plants</th>
<th>Dead plants</th>
<th>Missed plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Woodlands</td>
<td>~190</td>
<td>~46</td>
<td>~90</td>
<td>~9</td>
</tr>
<tr>
<td>Voortrekkerhoogte</td>
<td>~99</td>
<td>~20</td>
<td>~16</td>
<td>~2</td>
</tr>
<tr>
<td>Pinehaven</td>
<td>~102</td>
<td>~15</td>
<td>~11</td>
<td>~2</td>
</tr>
</tbody>
</table>

Treatment of *C. agatiflora* with imazapyr seems laborious and time consuming. This led to comparing imazapyr (Chopper) with glyphosate (Roundup) to see which one of the two herbicides is more efficient in controlling this species. Glyphosate is a broad-spectrum herbicide (Duke and Powles, 2008) and accounts for 11% of herbicide sales in the world (Powles et al., 1997). It is also known as a non-selective herbicide with no soil activity, making it ideal for directed usage (Powles et al., 1998).

Table 10 shows the differences in the imazapyr treatment versus the glyphosate treatment at the Woodlands population in Pretoria East. After the first follow-up the same population of Woodlands was treated with the same concentration of glyphosate as was previously used with imazapyr. The use of glyphosate on this species seemed to require less effort as there was no cutting of stumps needed and no seedlings were uprooted. Populations were just sprayed on the leaves as the herbicide is absorbed through the foliage. All plants died after having been treated with glyphosate, even the non-targeted species. In total glyphosate treatment required less time to complete. The effects of both chopper (imazapyr) and roundup (glyphosate) are clearly shown in Fig. 23 at the Woodlands population. On the first photo of Fig. 23, a healthy population can be seen in the background and the next photo shows the complete removal of that population with the treatment of chopper. One month after the treatment some plants resprouted and seedlings emerged. Roundup treatment followed after one week on the remaining re-sprouts and seedlings (photo 4 of Fig. 23).
Table 10: Treatment comparison of imazapyr and glyphosate on *Crotalaria agatiflora* in Woodlands, Pretoria.

<table>
<thead>
<tr>
<th>Chopper treatment (Imazapyr)</th>
<th>Roundup (Glyphosate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S25°49'14.8&quot; E28°19'07.5&quot;</td>
<td>S25°49'14.8&quot; E28°19'07.5&quot;</td>
</tr>
<tr>
<td>100 mL of Herbicide into 1 L water</td>
<td>100 mL of herbicide into 1 L water</td>
</tr>
<tr>
<td>18 m X18 m (4 labourers, 3 hrs 20 min.)</td>
<td>18 m X18 m (4 labourers, 20 min.)</td>
</tr>
<tr>
<td>~ 114 individuals treated</td>
<td>All visible individuals treated</td>
</tr>
<tr>
<td>186 seedlings uprooted</td>
<td>No seedlings uprooted (sprayed)</td>
</tr>
</tbody>
</table>

Figure 23: Imazapyr (Chopper) treatment versus glyphosate (Roundup) treatment of the Woodlands population in Pretoria. Photo 1 is before and photo 2 is after treatment in the same position where population was situated behind the person in photo 1.
The South African National Biodiversity Institute Invasive Species Programme (SANBI-ISP) employs contractors to deal with the control and clearing of alien invasive species. During the 2012/2013 financial year, SANBI appointed a clearing team in Gauteng to clear *C. agatiflora*. The clearing effort was successful to varying degrees. It is therefore essential to ensure a sustained control effort and follow-up over a number of years to ensure that gains made are not lost through lack of follow-up and monitoring. Therefore private companies bid towards tendering for SANBI-ISP hoping to offer their professional clearing services. However, contractors can be very aggressive when estimating their service charges. These services can be very expensive. Table 11 shows an example of a quotation from one of the approved contractors that cleared *C. agatiflora* for 120 days in 2012/2013.

SANBI-ISP is funded by the Working for Water programme (WFW) of the Department of Environment and the Expanded Public Works Programme. SANBI has received an amount of R37.58 million of which R15.7 million has been budgeted for rapid response clearing of emerging weeds either by chemical or physical control mechanisms in the financial year 2013/2014. SANBI-ISP invited invasive alien clearing contractors in the Gauteng or North West Provinces to submit expressions of interest to clear *C. agatiflora* in areas surrounding Gauteng and the North West Province in South Africa. The maximum value of this contract was R125 844.57 and the project stretched from September 2013 to November 2013 (60 days). One contractor is expected to have five people and to clear every *C. agatiflora* population on record and off record and they are expected to record the newly discovered populations. Records of *C. agatiflora* in the North West and Gauteng noted for clearing are relatively small populations and do not exceed 100 individuals. The amount budgeted by WFW and SANBI-ISP shows that indeed the Invasive Species Programme is serious about the controlling and eradication of emerging weeds although contractors are not always diligent. They can be very expensive and sometimes leave some populations behind in the hope of getting follow up contracts to do a similar duty. It would therefore be ideal if SANBI-ISP trains and establishes its own clearing teams around the country as this will ensure that clearing is done properly and costs are lowered in the long term. From the own clearing of three populations in Gauteng as part of this study, it seems that clearing of the *C.*
agatiflora can cost far less than the contractors are currently charging SANBI-ISP, considering also the number of populations recorded and sizes of populations.

Table 11: Costs of controlling Crotalaria agatiflora in Gauteng (2012-2013 financial year).

<table>
<thead>
<tr>
<th>Category</th>
<th>Contractors costs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total wage costs</td>
<td>R 13 478.20</td>
</tr>
<tr>
<td>Capital build-up</td>
<td>R 2 110.68</td>
</tr>
<tr>
<td>Camping allowance</td>
<td>R4 500.00</td>
</tr>
<tr>
<td>Personal protective equipments</td>
<td>R 907.60</td>
</tr>
<tr>
<td>Tools and Equipment</td>
<td>R 546.80</td>
</tr>
<tr>
<td>Transport</td>
<td>R 11 399.40</td>
</tr>
<tr>
<td>Administration</td>
<td>R 1 140.00</td>
</tr>
<tr>
<td>Workmans compensation 3%</td>
<td>R 404.35</td>
</tr>
<tr>
<td>UIF 2%</td>
<td>R 269.56</td>
</tr>
<tr>
<td>Liability insurance 4%</td>
<td>R 539.13</td>
</tr>
<tr>
<td><strong>Sub-Total</strong></td>
<td><strong>R 35 295.71</strong></td>
</tr>
<tr>
<td>VAT (14%)</td>
<td>R 4 941.39</td>
</tr>
<tr>
<td><strong>TOTAL QUOTATION COSTS</strong></td>
<td><strong>R 40 237.10</strong></td>
</tr>
</tbody>
</table>
Chapter 4: General Conclusions

*Crotalaria agatiflora* has been recorded in seven provinces in South Africa since its first record dating back to 1921 in the Pretoria National Herbarium. More than 35 populations of this species have been reported (recorded and observed).

Sizes of populations agree with the number of seeds collected from the soil-stored seed bank as smaller populations produce small numbers of seeds and large numbers of seeds are produced in large populations. There is no lateral spread of seeds outside the canopy, thus no evidence of strong seed dispersal except in the case where the species grows near streams (Lowveld population) so that seeds can be transported by water flow. The number of seed produced is very high in two populations (Colbyn and Lowveld populations) whilst there seem to be relatively low to none in the Howick, Pinehaven and Faerie Glen populations. The seeds await favourable conditions for scarification and soil conditions to germinate and advance the population. They have a high viability and germination capacity.

*Crotalaria agatiflora* seems to be a slow but persistent invader and this is largely due to its seed coat dormancy and ability to re-sprout, especially after fire.

DNA sequencing and isozyme studies showed no genetic variation, which could be as a result of a single introduction of this species.

The climate modelling shows that *C. agatiflora* can potentially invade some parts of South Africa (Limpopo, Mpumalanga, Gauteng, North West, Eastern Cape, and the Western Cape). The invasion puts the indigenous plants at the risk of competing with the alien species.

The Australian Weed Risk Assessment Model indicates a high potential of invasiveness with a score of 17.

Mechanical control using imazypyr requires regular monitoring and follow-ups, whereas control using glyphosate seems to save time when compared to imazypyr but is non-selective and kills all plants on contact. This may have a negative impact
in conservation areas but is perhaps less important on roadsides and other disturbed places where *C. agatiflora* typically invades.

The very high score obtained in the weed risk assessment model seems to suggest that *C. agatiflora* should be moved to category 1b in the NEMBA legislature so that it can be controlled as part of a directed management plan by the South African National Biodiversity Institute-Invasive Species Programme.
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