Developing biological control agents for the management of the invasive tree *Robinia pseudoacacia*

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Abstract

Robinia pseudoacacia (Fabaceae) is a deciduous tree native to the Appalachian Mountains of North America but has become naturalised and invasive in other countries such as temperate North America, Europe, Australia, and Southern Africa. In South Africa the tree is classified as a category 1B invasive alien under the National Environmental Management Act (NEMBA), which stipulates the species requires some form of control as it has already caused extensive negative ecological and economic impacts. In the invaded range the tree creates monocultures that displace native species and spreads rapidly from suckering roots, making it a proficient invader. The South Africa plant prioritisation system suggests *R. pseudoacacia* is in the top three species which should be considered for classical weed biological control in South Africa. This thesis investigates which insects known to be associated with tree should be prioritised as candidate agents, as well as offering interesting insights into prioritising insects for weed biological control and using plant phylogenies and available literature to predict insect specificity.

To ensure that no candidate biological control agents were already present in South Africa as well as to prioritise which guilds of the tree to prioritise for potential biological control, pre-release surveys were conducted across nine sites where the tree has invaded South Africa. It was found that no insects from the native range of *R. pseudoacacia* were present in South Africa. Seed surveys revealed that generalist insects attack a sizable proportion (68 %) of the seeds on the trees. In combination with a low seed soil bank (15.8 %) this suggests that seed- feeding agents may be helpful, however, candidate agents which damage leaves should be prioritised due to *R. pseudoacacia* relying heavily upon vegetative reproduction and much less on sexual reproduction. Leaves may therefor reduce the spread of these invasive trees

The insect assemblages in the native range of *R. pseudoacacia* are well understood. In addition, several associated insects have unintentionally followed the tree on its global spread, where they are often regarded as pests. The third chapter is therefore aimed at prioritising the known insects associated with the tree in both the native and invaded range. Literature surveys and Harris (1973) prioritisation systems were used to prioritise close to 64 candidate biological control agents down to three foliage- feeding agents, namely *Odontota dorsalis* (Coleoptera, Chrysomelidae), *Macrosaccus robiniella* (Lepidoptera, Gracillariidae) and *Obolodiplosis robiniae* (Diptera, Cecidomyiidae).

To further prioritise the six selected agents in Chapter 4, species distribution were modelled with known climatic variables. This was done by using the species known occurrence localities, from both the native and where applicable invaded ranges, to identify which species that would best match with South Africa's climate. The study showed that *O. dorsalis* is best suited to survive in South Africa, followed by *O. robiniae* and then *M. robiniella.*

Collected data aided in the introduction of the prioritised *O. robiniae* into South African quarantine facilities. Unfortunately, despite several attempts, cultures could not be established, making conventional host specificity testing impossible. Thus, in Chapter 5 we aimed to determine the potential host range of the midge using information from experts in the field of galling insects, literature surveys, agricultural pest lists, and social science platforms coupled with native and invaded range surveys. The list of non-target species to consider as potential hosts was refined by developing phylogenetic trees of closely related Fabaceae that share the same distribution (native and invaded) as *R. pseudoacacia*. Through the available information gathered, and field surveys of these species, *O. robiniae* has not been shown to utilise any species, other than those from the Robinoid clade. In addition, the midge has also never been recorded on a number of closely related leguminous fodder and horticultural species growing in close proximity at high densities to *R. pseudoacacia* - suggesting negligible risk to South African growers of the same species. Host-specificity assessments through field surveys can be regarded as one of the best indicators of the ecological host range, however, this information is difficult to quantify and infrequently available, thus seldom used when determining the safety of a candidate biocontrol agent. In this unique study, using the extensive data collected we are able to show that *O. robiniae* would be potentially safe for release in South Africa. However, open field tests exposing closely related non-target plant species under natural and semi-natural conditions are planned in Switzerland over the next two years aiming to confirm these conclusions.

By identifying and prioritizing potential biological control agents, this research contributes to the development of a targeted and sustainable solution for managing *R. pseudoacacia* in South Africa. The economic implications of successful biological control include reduced costs associated with invasive species management and ecosystem restoration. Additionally, by mitigating the negative ecological impacts, the agricultural systems in affected regions stand to benefit from enhanced resilience and productivity. Furthermore, the study's approach of using plant phylogenies and available literature to predict insect specificity offers a valuable methodological contribution to the broader field of weed biological control. This methodology can be adapted and applied to other invasive species, providing a framework for efficient and informed decision-making in weed management strategies. In essence, this research not only addresses the specific challenges posed by *R. pseudoacacia* in South Africa but also provides a template for tackling similar issues in different geographical contexts, thereby contributing to global efforts in sustainable agriculture and environmental conservation.

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Publications arising from this thesis

- Chapter 2

Arthropod herbivory on black locust (*Robinia pseudoacacia* L.) in the northern and southern hemispheres: implications for biological control in South Africa.

Abigail Wolmarans, Gerald Chikowore, Grant D. Martin

- *In progress*

- Furthermore, Chapters 3, 4 and 5 will be submitted to relevant journals.

Chapter 1. Introduction

1.1. Introduction

1.1.1. Invasion Biology

It is essential to preserve functioning ecosystems (Zhang *et al*., 2007). They provide ecosystem services that are defined as "the conditions and processes through which natural ecosystems, and the species that make them up, sustain and fulfil human life" and include, among others, soil fertility and pollination (Lawton, 1998; Zhang *et al*., 2007). Invasive alien species, primarily weeds, threaten these ecosystems and their natural habitat and biodiversity (van Wilgen *et al*., 2001; Rouget *et al*., 2002; Paterson *et al*., 2011; Paterson *et al*., 2019). When healthy ecosystems are threatened, natural biodiversity decreases and it reduces the efficacy of ecosystem services, in turn negatively impacting the economy and thereby affecting society (Alvarez & Cushman, 2002; Pimentel *et al*., 2005; Driesche *et al*., 2010; Pyšek *et al*., 2012; Prior *et al*., 2018). According to Mcfadyen (1998), alien plant species are the most significant invasive organism and are considered to have the highest negative impact economically and environmentally.

For decades, both intentionally and unintentionally, plant species have been imported outside of their native ranges; nevertheless, only a small number of these taxa are able to endure and reproduce (Richardson, 2000). When alien populations reproduce successfully, usually close to the parent plant, without intervention by people, they become naturalised. These plants are not yet considered invasive as they do not necessarily invade or negatively impact the environment (Richardson *et al*., 2000). Once a species spreads outside of the range it was introduced into, overcomes dispersal barriers, survives among other species in the new environment and reproduces within it, it becomes invasive (Baker, 1974; Richardson *et al*., 2000). Invaded regions are usually man-made disturbed areas consisting of semi-natural communities (Baker, 1974; Richardson & van Wilgen, 2004). An invading taxon would have to overcome resistance by native biota if it were to invade undisturbed communities (Richardson *et al*., 2000). Successful species will then be able to out compete native species and alter ecosystem functioning and processes, with the potential to create dense populations in natural habitats (Vilà *et al*., 2011; Pyšek *et al*., 2012; Staska *et al*., 2014).

It is important to consider the traits that make species successful invaders and to determine why some populations are more prone to invasion (Kolar & Lodge, 2001; Van Kleunen *et al*., 2015; Kwong *et al*., 2017). It enables ecologists to understand invasive plant dynamics and in doing so they are able to predict future invasions and prevent them (Kwong *et al*., 2017). It is also important when considering management strategies to target characteristics that ensure the success of the species (Blumenthal, 2005; Pyšek & Richardson, 2007; Van Kleunen, *et al*., 2015; Kwong *et al*., 2017).

The potential of a weed to become invasive due to specialized attributes has, however, been queried and is more likely context dependent, however, invasive alien species still differ either from native or other alien species with specific characteristics within certain contexts (Van Kleunen *et al*., 2015). Characteristics of successful invaders usually include rapid growth, higher seed output and better utilization of nutrients, therefore, reaching reproductive maturity quicker. They may also reproduce sexually and asexually, have high phenotypic plasticity and effective long- and short-distance seed dispersal mechanisms as well as seeds with great longevity (Baker, 1974; Kwong *et al*., 2017). They also have the ability to compete interspecifically and have higher tolerances to environmental stressors (Baker, 1974; Pyšek & Richardson, 2007; Van Kleunen *et al*., 2015; Kwong *et al*.,2017). Either these characteristics are a product of rapid evolution or they are inherent within a species, pre-adapting it to being a successful invader (Baker, 1974; Müller-Schärer *et al*., 2004; Kwong *et al*., 2017). Intrinsic factors alone do not guarantee a successful invasion. Extrinsic factors such as environmental disturbance, the availability of resources, climate and the competitiveness of native species also play a role (Shea & Chesson, 2002; Kwong *et al*., 2017). This has been evident in cases involving Australian acacias. They have many traits that are beneficial to humans, for example, their seeds, foliage, bark and wood have been used as livestock fodder for centuries (Richardson *et al*., 2011). This has resulted in Australian acacias being used and planted all over the world and in many cases they have become invasive because they are able to flourish in arid or degraded regions and grow unhindered in nutrient-poor soil (Richardson *et al*., 2011).

1.1.2. Trees as invasive alien species

It is well known that trees benefit people in various ways, whether through air purification, promoting the economy with natural resources or providing habitat for other organisms to name a few (Van Wilgen & Richardson, 2014; Turner-Skoff & Cavender, 2019). It comes as no surprise that trees have been distributed from their natural ranges in the past few centauries to countries all over the world (Rejmánek, 2014; Van Wilgen & Richardson, 2014). However, it was not until recently that they were considered to be potentially invasive and detrimental to humans (Rejmánek & Richardson, 2013). Introduced trees and shrubs are now a prominent feature on invasive alien lists and are among the top-ranked damaging plants. At least 20 percent of the best-studied invasive species are woody plants (Pyšek & Richardson, 2007; Van Wilgen & Richardson, 2014).

Managing invasive trees proves challenging as the majority of woody plants were planted intentionally and are still used commercially (Richardson, 1998; Rouget *et al*., 2002). Invasive research then creates many cases of conflict between natural resource management and researchers (Rouget *et al*., 2002; Van Wilgen & Richardson, 2014). Different types of conflict can arise from these situations regarding alien trees and may include conflicts of interest, conflicts of process or conflicts of information (Young *et al*., 2010; Van Wilgen & Richardson, 2014). Examples include the conflict between timber plantation owners and conservationists (Van Wilgen & Richardson, 2014), between those who encourage the use of trees to promote rural development and those who don't (Cohen, 2005) and the conflict between those who believe in planting trees to combat climate change and those who wish to reduce the amount of water consumed by trees (Jackson *et al*., 2005; Van Wilgen & Richardson, 2014). Conflict of responsibility also often arises between owners of land adjacent to plantations that suffer the consequences when invasive trees escape (Van Wilgen & Richardson, 2014).

According to van Wilgen and Richardson (2014), management outcomes should always be to "maximise benefits and minimise harm" but over time values and opinions have changed and some of the negative aspects of alien trees now exceed the benefits. To achieve the most beneficial outcome, especially in South Africa, management strategies will have to make trade-offs that will be in the interest of most people (van Wilgen & Richardson, 2014).

1.2. Management methods for invasive alien plants

Damage done to the environment and the economy by invasive weeds are enormous (Mcfadyen, 1998) but it is possible to reverse or at least slow plant invasions and to restore infested areas to healthy ecosystems with native species (Tu *et al*., 2001). This is possible by certain control methods to manage invasive plants (Tu *et al*., 2001). When it comes to allocating resources for management, it is important to understand the biology of the target invasive species as control is often quite costly and time consuming (Müller-Schärer & Schaffner, 2008).

1.2.1. Chemical control of weeds

Using registered herbicides with precise target sites, chemical control is a common and occasionally the least expensive strategy for getting rid of unwanted plants. (Owen & Powels, 2013; Martin, 2019). The target site in plants is where the herbicide binds to and in so doing, disrupts or alters metabolic processes. These target sites are usually enzymes and herbicides therefore stop or slow enzyme functions (Tu *et al*., 2001). Herbicides that use non-enzymatic target sites rather interact with photosynthesis by blocking or accepting electrons, thereby inhibiting photosynthesis. Others affect the production of aliphatic amino acids or disrupt cellular membranes (Tu *et al*., 2001). Herbicides have been used as early as 1941 and already made up 47 % of the world's agrochemical sales in the 90s (Quastel, 1950; Mcfadyen, 1998).

There are various methods of chemical control (Table 1.1.), with specific advantages and disadvantages concerning non-target species (Tu *et al*., 2001).

Table 1.1. Methods of chemical control of unwanted plants

Chemical control method	**Description**
Foliar application	For this procedure to work, the plant's leaves or stems must come into direct contact with the herbicide. Different application tools are used, but many of them can also harm plants that are not the intended targets (Tu *et al*., 2001).
Basal bark	Using this technique, herbicide is sprayed around the trunk of the target plant. Herbicides with ester formulations are typically applied to young trees with smooth bark (Tu *et al*., 2001).
Frill	This technique works best when holes are drilled into the trees' thick trunks, followed by an infusion of herbicide. Other techniques involve making a cut with a sharp instrument like a knife, saw, or axe before coating the cut with herbicide (Tu *et al*., 2001).
Injection	In this method, herbicides are injected into the stems either by specialized equipment or by hand with a needle and syringe. It is a powerful technique with no non-target consequences (Tu *et al*., 2001).
Cut-stump	For woody plants that resprout after being cut, this method works well. Herbicide is sprayed onto the inner bark of the stump after the tree or shrub has been felled. After treatment, some trees can

	sprout anew, necessitating monitoring and more treatment (Tu *et al.*, 2001).

Despite their success, herbicides do have adverse effects. There is toxicity to nearby non-target species, they leach toxins into the environment and the target species can become resistant (Tu *et al.*, 2001; Powles & Yu, 2010). Chemical control can also be extremely costly, difficult to use in native vegetation and sometimes unsuccessful in achieving broad-based control for many key weeds (Briese, 2004; Sutton, 2019).

In South Africa herbicides were not used predominantly in the forestry sector until the late 1980s (Little *et al.*, 2006). Unwanted vegetation was removed by hand or by hand-held implements but herbicides during this time provided the prospect of consistently reducing cost and increasing efficiency (Little *et al.*, 2006). Acknowledging the fact that herbicides may have negative effects on the environment, forest owners began investing in research initiatives to reduce the use of herbicides without compromising productivity, known as integrated management of vegetation (Little *et al.*, 2006). According to Dyer and Little (2002) there may be issues to take note of when implementing integrated management technology in South Africa. These include the resistance to change (Dyer & Little, 2002), unrealistic expectations (Dyer & Little, 2002), the inability to use or understand the technology (Dyer & Little, 2002; Fanadzo, 2007) and when the additional resources are seen to outweigh the economic return (Dyer & Little, 2002). Other methods of control therefore need to be investigated.

1.2.2. Mechanical control of weeds

The process of physically removing undesirable plants is known as mechanical control, and can vary from clearing large areas with machinery to manually uprooting seedlings and weeds (Bell & Lehman, 2005; van Wilgen *et al.*, 2001). Some techniques work by increasing the temperature of the soil, eliminating light or depriving the plant of carbon dioxide or oxygen (Tu *et al.*, 2001). Mechanical control is also considered either selective or non-selective. Selective techniques are meant not to impact non-target plants while non-selective methods affect the entire area that is being treated. It is possible to control or even eradicate certain weeds if mechanical control methods are applied at an ideal time and magnitude (Elsner & Gooden, 2010). There are various methods of mechanical control, which are briefly summarised in Table 1.2.

Table 1.2. Methods of mechanical control of unwanted plants

Mechanical control method	**Description**
Weed pulling	The weed's eradication from the environment by uprooting it. Most successful when all or the majority of the root system is eliminated (Gous, 1997; Tu *et al.*, 2001).
Mulching	Mulch is a substance that is spread on the ground and is seen as being reasonably easy and affordable in comparison to other ways. By blocking light and creating a physical barrier to stop their emergence, mulching smothers weeds (Tu *et al.*, 2001).
Soil sterilization/solarisation	This method for getting rid of weeds involves heat in the form of steam or solar energy. Although steam is injected into the soil, the process is expensive because it uses a lot of water. Solarization is the process of covering tilled, wet soil with a big, clear plastic sheet. Solar radiation heats the plastic, preventing the weed seeds from sprouting (Fouche *et al.*, 2000).
Mowing	Mowing reduces weed growth and reduces weed seed distribution by removing the top section of the weed (Elsner & Gooden, 2010). Many weed species lose vigour when biomass is repeatedly

	removed, hence this method is typically used in conjunction with other weed control techniques (Tu *et al*., 2001).
Tillage (cultivation)	Tillage is the process of turning the soil over. When dealing with crops, this technique is more frequently employed in agriculture (Rao *et al*., 2000). Tillage can be done on a small scale using hand-operated rotary tillers or on a big scale using ploughs mounted on tractors (Elsner & Gooden, 2010).
Flooding	By saturating the area to be cleared of weeds with water, this technique kills unwanted plants using an anoxic environment. However, it is inappropriate for regions with a meagre supply of water (Bell & Lehman, 2005).
Burning	Burning gets rid of weeds easily but might cause the dormant seed bank to reactivate, necessitating the use of additional mechanical techniques to fully regulate the situation (Holmes *et al*., 2008).

When huge areas are managed by specialized machinery or equipment, mechanical control alters the environment. By eliminating invasive alien plants, allowing native vegetation to re-establish, or by creating niches and encouraging the establishment of additional weeds, this will either have good or negative effects on the area. (Elsner & Gooden, 2010). Mechanical control frequently affects species that are not the target and can become very costly without achieving the desired control, resulting in wasteful expenses (Van Wilgen & Richardson, 2014).

In South Africa water hyacinth is removed mechanically and is considered the initial step of control (Hill, 2003). The process involves the use of rakes and pitchforks to remove the plant from the water and is considered effective in areas smaller than one hectare (Hill, 2003). If larger areas need to be cleared mechanical control is impractical and too expensive and often herbicides will be used (Hill & Olckers, 2000; Ueckermann & Hill, 2001; Hill, 2003) . This too has been unsuccessful as a follow-up regime is necessary and often neglected (Ueckermann & Hill, 2001; Hill, 2003;). Mechanical and chemical controls are therefore considered short-term solutions and more sustainable and cost effective means of control are required (Hill, 2003).

1.2.3. Biological control of weeds

Biological control is a sustainable and environmentally friendly approach employed to manage invasive alien plants in their invaded areas, mirroring conditions in their native environments. This strategy involves the introduction of natural enemies, termed biological control agents, from the plant's native range (Mcfadyn 1998; Driesche et al., 2010; van Wilgen, 2016). These agents, which can encompass insects with diverse feeding guilds, mites, herbivores, and pathogens, are selected for their high specificity resulting from co-evolution with the invasive plant species.

Importantly, before releasing biological control agents, rigorous assessments are conducted to ensure their safety and absence of non-target effects, particularly towards economically significant plants (Wapshere, 1974). The success of biological control programs hinges on the ability to reduce the abundance and vigor of the invasive plant, rather than complete eradication. The ultimate goal is to diminish its competitive advantage over native vegetation (DFFE, n.d.).

Post-release, an integral aspect of biological control is the ongoing assessment of the impact of the introduced agents. This evaluation involves monitoring the agents' effectiveness in suppressing the target invasive plant population and their potential impact on non-target species. Regular assessments help refine and optimize the biological control program, ensuring its long-term success (van Wilgen, 2016).

Furthermore, the establishment of biological control agents is a crucial phase in the process. It involves facilitating the agent's adaptation to the new environment and ensuring its sustained presence to exert continuous pressure on the invasive plant population (van Wilgen, 2016). The establishment phase may require ongoing monitoring, research, and adaptive management strategies to address any unforeseen challenges and enhance the overall effectiveness of the biological control program (van Wilgen, 2016).

1.2.3.1. Types of biological control

There are three main methods of biological control: classical (importation), which entails the introduction of a pest's natural enemy; inductive (augmentation), which employs a large population of natural enemies to quickly eradicate pests; and inoculative (conservation), which involves the regular reestablishment of natural enemies (Tu *et al*., 2001). These types of biological control are briefly discussed in Table 1.3.

Table 1.3. Types of biological control for unwanted plants

Type of biological control	**Description**
Classical (importation)	This method of biological management entails bringing a pest's natural enemies to a location where they do not already exist (Tu *et al*., 2001). The agent will have better control if it has temporal persistence. This allows it to sustain its population even while the target species is temporarily absent, and if it is an opportunistic forager, will allow it to quickly take advantage of a pest population (Follett *et al*., 2000).
Inductive (augmentation)	In an effort to quickly control a potentially devastating weed and solve a problem that has already developed, large numbers of insects are released. Augmentation is dependent on the specifics of how each pest and control agent interacts and can be useful but is not always successful (Wright, 1995)
Inoculative (conservation)	When naturally occurring, populations are increased by adding additional natural enemies to a certain area. To establish longer-

term control and maintain the pest at a low level, small numbers of the control agents are periodically released to allow them to multiply. This type of biological control emphasizes prevention over treatment (Wright, 1995).

1.2.3.2. History of biological control programmes for weeds

Use of the cochineal mealybug, *Dactylopius ceylonicus* Green (Dactylopiidae), against the cactus *Opuntia monacantha,* Haw. (Cactaceae) in 1863 (India) and 1865 (Sri Lanka) is the earliest record of the intentional use of biological control against weeds (McFayden & Willson, 1997). In 1902 the first significant programme of classical biological control was launched in an attempt to control *Lantana camara* L. (Verbenaceae) in Hawaii. It involved importing agents from their native range in Mexico and twenty-three insect species were shipped to Hawaii. In the end, eight insects, from the fourteen deemed safe for release, achieved control over the weed in most areas (McFayden & Willson, 1997). Between 1912 and 1914, *D. ceylonicus* was again used as a biological control agent and was shipped to Australia. *Opuntia* species cacti had become so problematic that valuable land could no longer be used. The cacti had originally been planted as drought fodder, ornamental plants and hedges in 1788 but progressively expanded into agricultural fields and pastures (Sternberg, 2020). Five insects were introduced during this time and successfully controlled *O. monacantha*, however, *Opuntia stricta* Haw. (Cactaceae) remained established (McFayden & Willson, 1997). Later, in 1920, the Commonwealth Prickly Pear Board was established with the sole purpose of controlling prickly pears by means of biological control. After extensive research in the native ranges of the prickly pear, Mexico, and southern United States, 48 insect species were imported to Australia. In 1925, along with 12 other released insect species, the moth *Cactoblastis cactorum* Berg. (Lepidoptera: Pyralidae) was imported from Argentina and by 1933 it had controlled all of the most cactus-infested areas in Australia (McFaydyen & Wilson, 1997; Goolsby *et al.*, 2020). The success led to other countries following suite and the moth was introduced to South Africa, the West Indies and some other smaller countries (McFayden & Willson, 1997; Sternberg, 2020).

1.2.3.2. Safety and success of biological control

Biological control is often viewed as a controversial topic as it may seem too high a risk by some, but others support the management option as it has known benefits to the environment (Briese, 2005; de Lange & van Wilgen, 2010). Risks include non-target attack on native species by biological control agents and the fact that these agents become permanent in the ecosystems they're released in (Mcfadyen, 1998). This either leads to excessively stringent protocols related to the release of agents or it results in total avoidance of biological control (de Lange & van Wilgen, 2010; Sheppard *et al*., 2006).

The argument for biological control is that it is considered the most environmentally friendly approach, as it does not leach impurities into the environment (Hill *et al*., 2000; Briese, 2005; Clewley *et al*., 2012; DEA, 2016). Biological control is often favoured above chemical control as herbicides have the potential to be harmful to other organisms, including humans, especially if they enter water supplies (Tu *et al*., 2001; de Lange & van Wilgen, 2010; DFFE, n.d.). Another advantage is that it has the potential to be highly specific (less than 1 % have had negative impacts globally) and is effective over large areas with little or no cost after the initial release of the insects (Mcfadyen, 1998; Fowler *et al*., 2000; de Lange & van Wilgen, 2010; Clewley *et al*., 2012; Suckling & Sforza, 2014; van Wilgen, 2016; Hinz *et al*., 2019). Economically the estimated cost to benefit ratio of biological control is 8:1 up to 376:1 but is dependent on species per species basis (de Lange & van Wilgen, 2010). It is also most likely to be the permanent solution to controlling invasive alien plants compared to other control methods, as the insects are often self-sustaining (Mcfadyen, 1998; Hill *et al*., 2000; Moran, *et al*., 2005; Clewley *et al*., 2012; DEA, 2016). The fact that it does not disturb soil or eliminate all the invasive plants at once is also beneficial as other invaders will not be occupying the area after control but allows natural vegetation to return naturally over time (DFFE, n.d.).

Opponents of biological control argue that, however small, it still has a risk factor and outcomes cannot be accurately predicted or that benefits might not compensate environmental costs. Examples such as the unintentional impacts on food webs or on non-target species, are often used as support (van Klinken & Raghu, 2006; de Lange & van Wilgen, 2010; van Klinken *et al*., 2016). Due to previous oversights, approaches to controlling invasive plants are becoming more and more cautious and often result in agents not being released at all (Briese, 2005; Sheppard *et al*., 2006). This is potentially more problematic as chemical and mechanical control methods are both expensive and unsustainable (de Lange & van Wilgen, 2010). It can also take decades for agents to establish and show results and sometimes they do not establish at all or establish with little or no effect on the target weed. Some would argue that even if they're established, the weed is never completely eradicated (Tu *et al*., 2001; van Klinken *et al*., 2016).

The safety of biological control is summarised in the Quarterly Review of Biology – "How Safe Is Weed Biological Control?" (Hinz *et al*., 2019), by addressing the following points: non-target attack went down from 18.2 % in the 1960s to 9.9 % from 1991 to 2008. This suggests that protocols to determine a biological control's host range have improved and that stricter import regulations have been set in place (Hinz *et al*., 2019). During pre-release trials, it is important to select plant species that will give the most accurate results when testing for non-target attack (Hinz *et al*., 2019). Worldwide there were fewer than 1 % false negative predictions made based on pre-release trials in intentional releases (Hinz *et al*., 2019). Of all international releases less than 1 % have the potential for non-target attack (Hinz *et al*., 2019). In determining a biological control agent's environmental safety post release, the field host range in its native range needs to be recognised as a vital aspect (Hinz *et al*., 2019).

1.2.3.3. Selecting biological control agents and host specificity testing

During early biological control programmes, the process involving the selection of agents and host specificity testing was not a priority. Researchers in the field would base assumptions on observations and would consider an insect safe for release if it was seen feeding only on the target weed (Briese, 2004; Julien & White, 1997). As a result, several species were introduced into countries without a full understanding of the host range. As such, some of the instances of non-target impacts by biocontrol agents are because suitable host range testing was not conducted (Paynter *et al*., 2004). Today, biocontrol releases are governed by very stringent regulations that govern all steps of a biocontrol programme. It is worth noting that a number of safe and effective biocontrol agents that were released in the past would not be approved presently for release due to the stricter regulatory regimes (Paynter *et al*. 2015; Hinz *et al*. 2019). Today biological control projects are based on a strong ecological framework and generally consist of six phases. They are: studying the target weed's ecology, exploration and pre-release surveys; prioritising biological control agents; evaluating the potential they have for biological control; host-specificity testing on closely related native plants, economically important or ornamental plants; mass rearing and release of the biological control agent; and evaluating and monitoring the agent in post-release surveys (Tu *et al*., 2001; Briese, 2004; Van Klinken & Raghu, 2006).

There are some considerations to take note of when it comes to the collection of potential agents. Selecting the optimal area within the weed's native range is important for establishment of the agent in the new introduced range. This means that an area that is Eco climatically similar to the weed's invaded range needs to be targeted (Briese, 2004; Müller-Schärer & Schaffner, 2008). Ecological Niche Models (ENMs) are used to identify these areas and they have been successful in identifying potential biological control agents for South African grasses that are invasive in Australia (Sutton, 2019). Phytophage diversity is also important when researching agents. The origin of the weed should be considered, and surveys should be concentrated on these evolutionary centres, as the potential agent would have the longest ancestral association with the weed. This means that the potential agent would have the greatest opportunity for evolution and specialisation in that area (Briese, 2004). Targeting populations in the native range with the closest genetic makeup to the weed in the invaded range would also be important when sampling potential agents (Briese, 2004; Sutton *et al*., 2017). For example, genetic matching identified that the problematic African tulip tree, *Spathodea campanulata* Beauv. (Bignoniaceae), which is invasive in the Pacific Islands, was genetically more similar to populations in their native range in West Africa than to those in Central/East Africa and that sampling efforts should be focused there (Sutton *et al.*, 2017).

Identifying the management objectives for biological control influences the selection of potential agents and becomes the benchmark for success, although success will also depend on the invasive plant species and environmental context (van Klinken & Raghu, 2006). It's possible that biological control can alter growth rate or cause individual mortality but not cause a reduction in population size or structure (Sheppard *et al.*, 2003; van Klinken and Raghu, 2006). Similarly, seed-feeding control agents may reduce seed production but not the density of non-seed-limited populations (Crawley, 1989; Van Klinken & Raghu, 2006). Sometimes biological control can reduce the density of aquatic weeds without restoring ecological function, for example the restoration of rivers (Van Klinken & Raghu, 2006). Understanding ecological processes and what the expectation for biological control is will define the level of damage that is required and will therefore assist in prioritising a biological control agent (van Klinken & Raghu, 2006).

Prioritization can follow two types of approaches. The first is thought to be more cost-effective by pushing resources into the introduction, testing and release of large numbers of the new control agents to maximise the chance of finding the right one. The second is more focused on the ecology of insect-plant relationships and concentrating resources on a smaller number of specific biological control agents that are limited to certain parts of the weed's life cycle (Briese, 2004).

There have also been attempts to formulate more objective methods of choosing agents using scoring systems or models (van Klinken & Raghu, 2006). These scoring systems are based on ecological theories from past biological control attempts, experimental data and experience and have been proven to be useful (Crawley, 1989; Sheppard *et al.*, 2003; van Klinken & Raghu, 2006). Their application however risks being oversimplified and neglecting valuable agents by being prone to counter argument and counter example. Specifically, comparing the climate of native and invaded habitats, even if they seem similar, reveals significant ecological differences rather than being equivalent. (van Klinken *et al.*, 2002; van Klinken & Raghu, 2006). Most of these types of approaches are untested and should be viewed with caution (van Klinken & Raghu, 2006). The criteria used in these methods are based on level of host specifity, distribution, phenology of attack and size (van Klinken and Raghu, 2006). The downfall is often that most of these criteira put more focus on agent traits and not enough on the weed's traits (van Klinken & Raghu, 2006). A more popular approach is by using ecological modeling and using "agent-weed" population dynamics to determine potential agents (van Klinken & Raghu, 2006). Population dynamics of an invasive plant, studied within native and invaded ranges, can help identify the part of its life cycle that no longer has ecological constraints, causing it to become invasive and therefore ideal for the prioritisation of a biological control agent (Sheppard *et al.*, 2003). Comparing insect herbivores found in the weed's native range to insects in the invaded range also clarifies which insects are needed for control. If the weed is able to spread with some insects feeding on certain guilds, those parts of the plant not attacked should be targeted with control agents (Briese, 2004). Studies on plant-insect interactions and weed ecology provide insight into whether particular types of agents, such as seed-feeders, can effectively influence plant populations or whether a particular agent's life-history aspects can maintain sufficient control (Briese, 2004). Effective prioritisation of agents therefore increases the likelihood of a successful biological control programme by increasing the probability that effective agents are released (Sheppard *et al*., 2003; van Klinken & Raghu, 2006).

These aspects can be considered filters in the agent selection process. Beginning with exploration and documentation of fauna in the native range through survey work. For agent selection, detailed knowledge on potential agents that are available can be beneficial (van Klinken & Raghu, 2006). The second is determining the management objectives and selecting a suitable agent that causes the desired damage to the individual weed or population considering time and types of damage (van Klinken & Raghu, 2006). The third focuses on whether potential control agents will be able to reach high enough densities to make a difference in the introduced range (van Klinken & Raghu, 2006). The final compares insects to pathogens to determine what similarities and differences they have and whether there are synergies that can be beneficial (van Klinken & Raghu, 2006).

1.2.3.4. Host Range Testing for biological control

Once an agent has been selected, the next crucial step is host-specificity testing to ensure that non-target organisms will not be affected (Heard, 2002; Briese, 2004; Heard & Van Klinken, 2004; Schaffner *et al*., 2018). Host-specificity testing is usually the biggest hindrance or challenge to biological control as it is the most time consuming process and can become costly when technical support and facilities are considered (van Klinken & Raghu, 2006; Liu *et al*., 2019a). Agents will be tested according to host-ranges in a quarantine setting until deemed safe for release (Briese, 2004; Withers *et al*., 2018). Host-ranges refer to plants that insects would feed on under laboratory conditions and plants that were observed to be their hosts naturally (Briese, 2004). According to Schaffner (2001) there are two types of host-ranges, namely fundamental as opposed to realised host-ranges. Fundamental host-ranges refer to all plants on which the insect herbivore is capable of feeding, while realised host ranges refer to how the insect herbivore reacts under particular conditions, such as certain limitations (Heard & van Klinken, 2004). The realised host-range is considered the more important of the two because it is the predicted host-range (Goolsby *et al*., 2020).

Plant selection for host-specificity testing is done by determining relatedness to the target weed by degree of phylogenetic separation in order to understand what an agent is likely to attack rather than what it would not attack (Briese, 2005; Dube *et al*., 2019). For example, an agent released for the control of a *Lantana* spp. in Australia had attacked a non-target species of exotic ornamental plant in the Verbenaceae family that had not been tested for host-specificity. If phylogeny had been used researchers would have been informed about several closely related genera within similar phylogenetic distance of *Lantana* that were at risk and should be included in host range tests (Briese, 2005).

Experiments for host-specificity then examine aspects of host selection by agents according to six criteria. These include adult feeding, oviposition, larval feeding, larval development, adult longevity, and fecundity. To simulate natural conditions outside of quarantine, tests are then done in the field (Heard, 2002). Host-specificity tests are divided into two approaches: choice tests and no-choice tests. During no-choice tests insects are positioned on selected plant species within different containers which vary according to what suits the insect such as petri dishes, cages, jars or water- filled containers (Heard, 2002; Sheppard *et al.*, 2005). During choice tests insects are placed in the same suited containers with the selected plant species as well as the target weed, which acts as the control (Heard, 2002; Sheppard *et al.*, 2005). Another test design, called the choice-minus-target test, is used when an insect is given a choice between two selected plant species in the absence of the target weed, therefore without a control (Heard, 2002; Sheppard *et al.*, 2005; Paynter *et al.*, 2015). No-Choice test results define the "fundamental host-range" when the target weed is unavailable (Heard, 2002; van Klinken & Edwards, 2002; Paynter *et al.*, 2015; Schaffner *et al.*, 2018) and are often more reliable as potential non-target hosts are more likely to be utilized when potential agents are "starved" compared to "satiated" (McEvoy, 1996; Sheppard *et al.*, 2005; Paynter *et al.*, 2015). Both of these situations are valid because they may occur naturally, although no-choice tests often reveal a host range larger than that known to be used in the native range (Heard, 2002; Schaffner *et al.*, 2018).

Larval development, adult longevity and fecundity tests can only be done using the no-choice test approach while oviposition, adult feeding and larval feeding can be done with either approaches (Heard, 2002; Sheppard *et al.*, 2005). Field surveys and open field tests are again restricted to choice tests (Heard, 2002). Oviposition test and larval development tests are often done together to assess the behavioural preference of the adults and the suitability of the plant for larval development (Heard, 2002; Paynter *et al.*, 2015). Sometimes oviposition will only be tested on plants that support larval development as some insects have a limited ability for dispersal and therefore larval development trials are most important for selected plants species where ovipostion takes place (Heard, 2002; Paynter *et al.*, 2015). It is important to note that oviposition and larval development tests are prone to give false positive results as adults may eventually "dump" eggs on the plants they are not able to develop on and larvae may have a larger host range than the plants available naturally. If no other tests are conducted this may result in the rejection of safe agents (Heard, 2002; Paynter *et al.*, 2015). It has been found that Lepidoptera and Hemiptera oviposition behaviour is difficult to obtain and therefore larval development and ovipositioning tests cannot be conducted simultaneously (Heard, 2002). Adult feeding tests are very often explored but trials are restricted to agents that feed destructively and therefore such tests on Cecidomyiidae for example are unnecessary as they do not feed at all or, for example, Lepidoptera, Bruchidae or Tephritidae that usually feed non-destructively (Heard, 2002). If larvae cannot develop on those plants, adult feeding is of little use as it will only result in minimal damage (Heard, 2002). However, public perception is very important and even cosmetic damage can result in safe agents not being released (Heard, 2002). For example *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae), an agent used against *Parthenium hysterophorus* L. (Asteraceae) was terminated as a control agent in India after a small amount of feeding on sunflowers (Heard, 2002). The adult fecundity test is done to ascertain whether selected plants can support egg production (both acceptability of the food and suitability for egg maturation), but is restricted to agents that depend on adult feeding for continued egg production (Heard, 2002). Adult longevity tests are usually done along with adult fecundity tests (Heard, 2002).

1.2.3.5. Biological control field trials

Selected test plants are placed in the native range of the agent during open-field tests. This may result in an increase in abundance of local agents. These results are more likely to reveal the realised host range of the agent as it encompasses all the aspects of host selection, especially when conducted without a cage (Heard, 2002; Sheppard *et al*., 2005; Paynter *et al*., 2015). Field surveys are very similar except that spatial arrangements and agent densities are not manipulated (Heard, 2002).

1.2.3.6. Biological control post release

After agents have been released, post-release surveys are necessary to quantify the success of the programme and to identify whether management targets have been reached. It is also useful to identify whether resources allocated to biological control were justified and to provide economic evaluation of the benefits of biological control (Morin *et al*., 2006; Hayes *et al*., 2013; Martin *et al*., 2018).

Although post-release evaluation should be done routinely, it's found that the research concerning post-release evaluation is still understudied (Schaffner *et al*., 2020). This may be due to policy makers being sceptical about continually putting funding into the biological control programme once it appears to have been successful (Schaffner *et al*., 2020). However, quantitative post-release evaluations remain a necessity as success of a programme faces certain obstacles even after a successful release and reduction of the target weed (Schaffner *et al*., 2020).

1.2.4. Biological Control in South Africa

In common with many other countries, thousands of alien plant species have been introduced into South Africa (Nel *et al*., 2004; Moran *et al*., 2013;). Some have become naturalized and do not need tending but others have become invasive (van Wilgen *et al*., 2001; Moran *et al*., 2013;). These weeds are at many different stages of invasion ranging from invasive plants that are already well established to others that are in the early phases of invasion (Nel *et al*., 2004). This leads to the need to control potential alien invasive species (van Wilgen *et al*., 2012). Many control strategies exist and they aim to reduce the risk of new introductions of invasive alien species, to mitigate the impact of existing weeds through effective control, and the establishment and management of legislation to guide implementation (van Wilgen *et al*., 2012).

South Africa is considered to be one of the most active countries when it comes to research and the implementation of biological control (Moran & Hoffmann, 2015). In 1913, the first successful agent in South Africa was the cochineal insect against the prickly pear cactus *O. monacantha* (Moran *et al*. 2013). According to Zachariades (2021), 93 biological control agents have since been released and established on 59 IAP species and include plant pathogens, mites and insects. At present, there are numerous cases of successful biological control in South Africa where no other means of control is necessary. These weeds include *Hakea sericea* Schrader (Proteaceae), the silky hakea controlled by numerous agents including gummosis disease, *Sesbania punicea* Benth. (Fabaceae) the red sesban controlled by three agents, *Acacia saligna* (Labill.) H.L.Wendl. (Fabaceae) the Port Jackson wattle controlled by gall rust fungus, *Harissa martini* (Harrisia cactus) controlled by the mealybug and *Opuntia stricta* Haw. (Cactaceae) the Australian pest pear controlled by a biotype of *Cochineal* (DFFE, n.d.).

1.2.5. Invasive trees and shrubs in South Africa

According to Rejmánek (2014), there are at least 434 invasive trees and 317 invasive shrubs in the global database of invasive trees and shrubs. They have different traits that have different negative impacts on ecosystems such as nitrogen fixation or higher growth rate (Mostert *et al*., 2017). For example, *A. saligna* has a competitive advantage over natural vegetation because it has a higher growth rate than native flora and can reach taller heights, resprouts quickly after fires and mechanical damage, has a large and dormant seed bank and has the ability to fix nitrogen (Mostert *et al*., 2017). Invasive trees and shrubs can also have negative effects on resident fauna (Clusella-trullas, 2016). Invasive trees and shrubs can impede essential functions for animals, such as movement and refuge-seeking, while also disrupting their food resources through changes in trophic interactions. (Clusella-trullas, 2016). Invasive trees and shrubs can also have a negative impact on water quality in South Africa (Chamier *et al*., 2012). This can occur when trees and shrubs increase in biomass above ground and alter evapotranspiration which then decreases surface runoff and ground water recharge (Chamier *et al*., 2012). Increased biomass from trees and shrubs that occur in areas where invasions are prominent can cause more intense fires that may lead to excessive erosion. These invasions are also the cause of reduced carrying capacity of pastures and range lands which then cannot support livestock and wildlife (Chamier *et al*., 2012).

In South Africa, some trees are effectively being supressed by biological control (Hoffmann & Moran, 1999; Hoffmann *et al*., 2002; Impson *et al*., 2011; Wilson *et al*., 2011). A number of these are Australian acacias and are under control. For example, *Trichilogaster acaciaelongifoliae* Frog. (Hymenoptera: Pteromalidae) the bud-galling wasp, which attacks the reproductive parts of the *Acacia longifolia* Andr. (Fabaceae), the long-leaved wattle and thereby supressing its invasive potential (Dennill & Donnelly, 1991). The Port Jackson wattle (*A. saligna*) is also under effective control by the gall-forming rust fungus, *Uromycladium tepperianum* (Uredinales: Pileolariaceae), by affecting its reproductive and vegetative growth (Impson *et al*., 2011). There has been successful biological control among other trees as well in South Africa. Three different biological control agents have brought the red sesban (*S. punicea*) under control (Hoffmann & Moran, 1999). The bud-feeding weevil, *Trichapion lativentre* Beguin-Billecocq (Coleoptera: Apionidae) is very effective at destroying buds on the tree and reduces pod production by at least 98 % (Hoffmann & Moran, 1999). *Rhyssomatus marginatus* Fahraeus (Coleoptera: Curculionidae) was the second insect to be introduced and is able to reduce seed production even more (Hoffmann & Moran, 1999), and thirdly *Neodiplogrammus quadrivittatus* Olivier (Coleoptera: Curculionidae), whose larvae create enough damage by boring into the trunks and stems of the tree that they are able to kill it (Hoffmann & Moran, 1998, 1999).

There are, however, many trees and shrubs in South Africa that are not being targeted by biological control and may not be managed effectively by chemical or mechanical control (DEA, 2015; Martin, 2019). These include species such as the honey locust, *Gleditsia triacanthos* L. (Fabaceae), hickory wattle, *Acacia implexa* Benth. (Fabaceae), red river gum, *Eucalyptus camaldulensis* Dehnh (Myrtaceae), weeping willow, *Salix babylonica* L. (Salicaceae), and the black locust, *Robinia pseudoacacia* L. (Fabaceae) to name a few (Henderson & Wilson, 2017). This study will focus on developing biological control options for *R. pseudoacacia*. It is not known if *R. pseudoacacia* will respond to biological control in the same manner *S. punicea* did as a tree from the same family, and little is known about seed dispersal and the dominant mode of spread in South Africa. It is also unknown how *R. pseudoacacia* drives other invasions where they are present.

1.3. Target Species: *Robinia pseudoacacia*

In Europe *Robinia pseudoacacia* (figure 1.1) is regarded as one of the top 20 weed targets for classical biological control from an ecological and socioeconomic perspective (Sheppard *et al*., 2006).

Figure 1.1. The target species, *Robinia pseudoacacia*

1.3.1. Taxonomy of *R. pseudoacia*

Robinia pseudoacacia (Fabaceae), commonly known as the black locust, false locust, and yellow locust or in Afrikaans, witakasia or valsakasia (ISSA, 2022). From the history and etymology *Robinia* honours Jean Robin (1550-1629), of Paris, gardener to Henri IV and Louis XIII of France, who received new plants from Canada. Because it resembles an acacia but is not one, the word pseudoacacia is directly translated as fake or false acacia. Early missionaries gave the tree the name "locust" because they thought it had supported St. John in the wilderness. However, it is an American tree, not a native of any other region in the world (Martin, 2019). *Robinia pseudoacacia* was discovered in 1607 at Jamestown by British colonists, who used the wood to build houses. It is one of four species and four presumed hybrids from the modern circumscription of the *Robinia* Genus L. (Isely & Peabody, 1984; Lavin & Sousa, 1995) all of which are native to North America (Cierjacks *et al*., 2013). *Robinia pseudoacacia* L., *Robinia hispida* L. and *Robinia viscosa* Vent (Fabaceae) are native to the Appalachian Mountains, south eastern region of North America and *Robinia neomexicana* A. Gray (Fabaceae) is native to the south western mountains of North America, adjacent to Mexico (Isely & Peabody, 1984).

1.3.2. Description of *R. pseudoacia*

Robinia pseudoacacia is a medium-sized hardwood deciduous tree (or sometimes large shrub) that is usually 12 - 25 m in length but potentially may reach 30 m or more (Henderson, 2001; ISSA, 2022). The bark is smooth on a young tree and becomes dark brown, thick, and deeply furrowed over time (figure 1.2.). These grooves have a hint of red/orange colouring and run vertically with the trunk, sometimes creating diamond patterns (Lavin & Sousa, 1995; Dirr, 1999; Henderson, 2001; ISSA, 2022). The twigs on the branches have paired stipules that are modified into spines and may grow up to 1.5 cm long (Stone, 2009).

Figure 1.2. The bark of *Robinia pseudoacacia* becomes dark brown, thick, and deeply furrowed over time

The tree's leaves are compound and imparipinnate, with 2 - 12 mostly opposite, sometimes alternate at maturity, paired leaflets on either side of the rachis, typically ending with an odd pinnate (figure 1.3.) (Lavin & Sousa, 1995; Cierjacks *et al*., 2013). The dark blue - green leaflets with a contrasting lighter underside are rounded, narrowly to widely elliptic with an abaxial surface glabrate to sericeous found with or without finely scattered tannin deposits (Lavin & Sousa, 1995). The leaves turn yellow in autumn before they drop just before winter and new leaves then appear quite late in spring (Lavin & Sousa, 1995; Henderson, 2001; ISSA, 2022). The tree grows upright with a straight trunk and forms an oval or rounded crown (Dirr, 1999; Henderson, 2001). Some parts of the plant are toxic, particularly the bark but also its leaves, stems, and seeds. Occasional instances of livestock and human poisoning have been reported (Cooper & Johnson, 1985; Veitch *et al*., 2010). Ingestion results in both gastrointestinal and neurological effects which are particularly acute in horses and may be fatal (Michigan Department of Natural Resources, 2012).

Figure 1.3. Leaflets of *Robinia pseudoacacia* trees are paired on either side of the rachis, typically ending with an odd pinnate

In its native range, *R. pseudoacacia* produces sprays of pendulous flowers from May to June after the leaves have fully developed (figure 1.4.) (Henderson, 2001). In South Africa the trees flower from September to November (ISSA, 2022). Within the *Robinia* genus, only *R. pseudoacacia* displays white flowers while the other species exhibit drooping pink flowers (Cierjacks *et al*., 2013). Racemes are typically 18 – 25 cm long consisting of many diadelphous (pea-like) flowers (Isely & Peabody 1984). Flowers are also exceptionally fragrant and produce large amounts of nectar (Huntley, 1990; Sheppard *et al*., 2006; Vítková *et al*., 2017a).

Figure 1.4. *Robinia pseudoacacia* produce white, pendulous, and exceptionally fragrant flowers

The fruit of *R. pseudoacacia* is a typical legume fruit, with oblong, flattened pods that ripen in the spring (figure1.5.). These pods are dark brown with a reddish tint, and several pods can be found on a single stalk. They are 5 to 12 cm long and contain 4 to 8 bean-shaped seeds (Cierjacks *et al*., 2013). When the seeds mature, they will have a strong, impenetrable coat, and the fruit will open on the tree in late October, scattering seeds, while the pod remains on the tree all winter (Huntley, 1990; Henderson, 2001).

Figure 1.5. The seed pods found on *Robinia pseudoacacia* trees year- round

1.3.3. Reproduction and dispersal of *R. pseudoacacia*

Robinia pseudoacacia is able to reproduce both sexually and asexually by means of seed dispersal and root suckering (Surles *et al*., 1990; Chang *et al*., 1998). When its flowers are in bloom, they are mostly pollinated by insects from the order Hymenoptera and are mostly bees (Surles *et al*. 1990; Huntley, 1990). Each flower is considered perfect as it has both stamens and a pistil but are physically separated to prevent self-pollination. Protogynous flowering also promotes out-crossing as the dominant mating method (Surles *et al*. 1990). Nevertheless, according to Yuan *et al*. (2013) variations in flower maturation can lead to self-fertilization by geitonogamous selfing. *Robinia pseudoacacia* trees then begin to produce seeds when they're approximately six years old, though seed production is at its best when the tree is between 15 and 40 years of age and continues until it reaches 60 (Huntley, 1990). It has been discovered that every 45 kg of fruit produces 7 to 15 kg of seed at a rate of 52 900 seeds per kg (Huntley, 1990). Previous research in its native range discovered a high level of genetic diversity, with the majority of the variation occurring within *R. pseudoacacia* populations.

Genetic divergence is most likely the result of populations drifting apart through time (Surles *et al.*, 1990; Chang *et al.*, 1998; Liesebach *et al.*, 2004; Gu *et al.*, 2010). Individual trees in monodominant forests can yield up to 12,000 seeds per m^2 and 640 to 2285 seeds per m^2 beneath single trees (Cseresnyés & Csontos, 2012). New populations, on the other hand, do not form as easily. Long-distance dispersal is mostly accomplished through human-related vectors such as the conveyance of soil containing seeds or roots, deliberate planting, or accidental transport into new locations (Von Der Lippe & Kowarik, 2008; Pyšek *et al.*, 2012). Their germination then depends on environmental conditions, bare soil being the most suited for seed development. In the native range it is found that the percentage that germinate is very low, varying between 3.5 % and 16.3 %, as the seeds have a strong protective coat and need scarification to germinate (Baskin *et al.*, 2000; Chytrý *et al.*, 2005; Vítková *et al.*, 2015b; Sádlo *et al.*, 2017). Long- distance spread by natural means is quite uncommon due to a somewhat heavy seed which will only disperse near the mother tree by wind and gravity. There are, however, some exceptions such as dispersal by water and it has been found that pods can be carried up to 1 200 m along river currents (Säumel & Kowarik, 2013). In Japan it was found that wind could blow seeds along surfaces covered in snow up to 67 m (Morimoto *et al.*, 2010) and dispersal has also been seen to be facilitated by endozoochory by birds and pigs (Cierjacks *et al.*, 2013). In this regard, *R. pseudoacacia* differs from other successful invasive plants that have colonized disturbed areas even far from the mother plant thanks to their high percentage germination and high dispersal ability by water, wind or birds (Perglová *et al.*, 2009; Vítková *et al.*, 2017b; Skálová *et al.*, 2019).

While some plants are clearly individuals, others form part of a collective organism. Many plants reproduce vegetatively by sending up shoots that form on underground roots. For example, the largest organism in the world may be a clone of connected and genetically identical aspen trees in North America. One such clone from the Wasatch Mountains is spread across 80 hectares (Nabors, 2003).

Similarly, *R. pseudoacacia* has the ability to reproduce by suckering and therefore by creating clones (Chang *et al*., 1998; Huntley, 1990; Kowarik, 2003; Lee *et al*., 2004; Redei *et al*., 2008; Ábri & Rédei, 2022). These clones might be at least several hundred years old, much older than the trees apparent today (Chang *et al*., 1998). In areas where *R. pseudoacacia* stands are not disturbed, they are able to spread locally up to 1 m per year by means of root elongation (Cierjacks *et al*., 2013; Pergl *et al*., 2016). According to Vítková *et al*. (2017a) *R. pseudoacacia* is extremely resistant to disturbance. In areas where they are disturbed, they create persistent buds on their branches, stems and roots allowing them to recover quickly. They regenerate quickly by suckering, and stem densities may even increase after mechanical damage (e.g., coppicing). They also form larger and stronger spines in areas where damage has occurred (figure 1.6.).

Figure 1.6. Spines (or thorns) become more prominent on *Robinia pseudoacacia* trees after disturbances.

These elements promote the regeneration of *R. pseudoacacia* stands, which expands and compacts the colonies, which eventually cover hundreds of square meters (figure 1.7.) (Pergl *et al*., 2016). Even trees over 70 years old develop root suckers, and when compared to seedlings of other species of the same age, they are substantially higher, grow faster, and achieve reproductive maturity earlier (Pergl *et al*., 2016; Vitková & Kolbek, 2010). In South Africa, *R. pseudoacacia* can spread quickly by seeds and suckering roots, displacing native plants, and forming monocultures (Humphrey *et al*., 2019b).

Figure 1.7. *Robinia pseudoacacia* trees can produce compact clonal colonies after mechanical control attempts.

1.3.4. Habitat and ecology of *R. pseudoacacia*

Robinia pseudoacacia is known to be one of the top ten neophytes with the broadest habitat range (Vítková *et al*., 2015a). It is a light-depending species and is able to disperse quickly through root sprouts and a fast growth rate. This makes it a pioneer species that is able to colonize a broad range of xeric to mesic habitats, including steep rocks or toxic man-made substrata (figure 1.8.) (Bartha *et al*., 2008; Boring & Swank, 1984; Cierjacks *et al*., 2013; Vítková & Kolbek, 2010). It is not restricted to any one soil type, but prefers damp, loam-type soils. It is most commonly seen in Cambisol with incipient soil formation. Cambisols predominate in temperate and boreal climates but are less common in the tropics and subtropics (Huntley, 1990).

Figure 1.8. *Robinia pseudoacacia* trees are able to colonize a broad range of habitats, ranging from urban environments to pristine areas.

It is also found in a wide range of environments

; though it favours mountainous areas with elevations less than 1 040 m (figure 1.9.) (Kleinbauer *et al*., 2010; Vtková & Kolbek, 2010; Vtková *et al*., 2015a). *Robinia pseudoacacia* exhibits optimal growth in a humid climate where rainfall ranges from 1 020 to 1 830 mm annually and where temperatures range from maximum 27 to 32 degrees °C in summer and minimum from -7 to 13 degrees °C and up in winter (Huntley, 1990). From seedlings, the tree is intolerant of shade, but vegetative offspring, which are still connected to the mother plant by the root system are considered to be more shade tolerant (Huntley, 1990; Knapp & Canham, 2000; Radtke *et al*., 2013).

Figure 1.9. *Robinia pseudoacacia* trees are mostly found in mountainous areas.

In disturbed areas it dominates early stages of regeneration and colonizes open sites in suburban wasteland, mining areas, abandoned fields and pastures, forest gaps or sites damaged by fire (Maringer *et al*., 2012; Cierjacks *et al*., 2013; Radtke *et al*., 2013; Pergl *et al*., 2016). The problem is that invasive species threaten the ecological integrity of natural areas by influencing community structure and function and by altering ecosystem processes (Ehrenfeld *et al*., 2001; Evans *et al*., 2001; Rice *et al*., 2004; Deneau, 2013). Numerous studies show the negative effects of *R. pseudoacacia* in its invaded range, for example, the fact that it forms specific plant communities, whose plant layer is markedly different from that in forest dominated by native trees. It changes the conditions under the canopy, which become more favourable for shade-tolerant and nitrophilous species (Vítková & Kolbek, 2010; Pergl *et al*., 2016). It also homogenises the tree composition of the spontaneously growing forest patches, showing a tendency toward forming pure pioneer stands which are highly different from native plant communities (Vítková & Kolbek, 2010; Benesperi *et al*., 2012; Sitzia *et al*., 2012) . This suggests that the slight homogenisation of the ground-layer species abundance may be linked to the less heterogeneous micro-site conditions under the *R. pseudoacacia* stands than under the native stands. In fact, the strong cover of *R. pseudoacacia* might imply a homogenous pattern of light reaching the ground layer (Rice *et al*., 2004; Sitzia *et al*., 2012).

It is possible that one of the strong effects *R. pseudoacacia* has on native vegetation, other than manipulating light intensity, is caused by increased nutrient availability associated with the nitrogen fixing ability of the symbiotic Rhizobium bacteria occurring in *R. pseudoacacia* root nodules (Ferrari & Wall, 2007). Symbiotic fixation is an important input for the nitrogen cycle in *R. pseudoacacia* stands, more important than litter mineralization or other sources (Tian *et al*., 2003; Williard *et al*., 2005; Liu *et al*., 2019). In its native range it can fix 33 to 75 kg Nitrogen ha^{-1} year^{-1}, with a particularly high capacity for N_2 fixation in early to intermediate stages of secondary succession (Boring & Swank, 1984; Vítková *et al*., 2015b). High amounts of fixed nitrogen may exceed the demand for plant nutrition in cases where *R. pseudoacacia* takes over in a region causing the excess nitrogen to accumulate in soil and to escape through leaching, runoff and denitrification (Buzhdygan *et al*., 2016). These processes may be followed by soil degradation and a significant decrease in the soil pH (Buzhdygan *et al*., 2016; Liu *et al*., 2022). Furthermore, frequent inundation of floodplains can cause nitrate contamination of river water followed by the pollution of ground water (Buzhdygan *et al*., 2016). It also has severe impacts on other plant communities (Rice *et al*., 2004). Invasion by a nitrogen-fixing species can supplement nitrogen stores and increase rates of nitrogen cycling and availability (Corbin & D'Antonio, 2012). Given that plant growth in most temperate, terrestrial systems is usually limited by nitrogen availability, nitrogen inputs caused by invasive nitrogen-fixers not only influence primary productivity, but may have cascading effects on successional patterns, community composition, and disturbance regimes (Evans *et al*. 2001). In addition, nitrogen supplementation may hinder efforts to restore native plant community and ecosystem function on previously disturbed sites as soil nitrogen levels remain elevated once *R. pseudoacacia* has been removed (Rice *et al*., 2004). Once established, *R. pseudoacacia* may even facilitate the spread of other exotic species (Hu *et al*., 2021).

1.3.5. Economic and Ecological Uses of *R. pseudoacacia*

Robinia pseudoacacia is often considered an economically important tree in its native and introduced range and therefore has a number of uses. It is a fast-growing tree producing valuable water and rot resistant timber which is considered the hardest wood in North America (Nicolescu *et al*., 2020). The wood is used for many purposes. Historically, it was important for vineyard poles, wine barrels, boats, roof shingles, and fence posts. Currently its wood is used for fencing and for making furniture, insulator pins, garden and playground equipment, mine timbers and treenails for ships (Alden, 1995; Bartha *et al*., 2008; Sádlo *et al*., 2017; Hillier & Lancaster, 2019; Nicolescu *et al*., 2020). *Robinia pseudoacacia* is also highly valued as firewood, especially for wood-burning stoves. It has a small amount of visible flame and smoke, burns slowly and has a high heat content similar to anthracite (Marcouiller & Anderson, 1970).

It is also a popular tree for street avenues and parks in urban areas as it tolerates high levels of air pollution, light intensity, salinity, low soil quality and drought (Vítková *et al*., 2017a; Hillier & Lancaster, 2019). Its fast growing nature, high yield, nitrogen fixing ability and the fact that it can grow densely even in acidic conditions also makes it popular for biomass production in short-rotation energy plantations (Alden, 1995; Redei *et al*., 2008; Sádlo *et al*., 2017). These attributes make *R. pseudoacacia* a favoured choice for erosion control, reclamation of strip mines, soil improvement, windbreaks, provision of leaf forage to animals, or facilitating increased shade as a nurse tree. (Vítková *et al*., 2017; Ciuvăț *et al*., 2022).

In its native range, *R. pseudoacacia* is well known as a honey tree because of its fragrant flowers and high nectar yield producing a good quality monofloral honey (Atanasov *et al*., 2022). Other than in North America, honey is produced from *R. pseudoacacia* mainly in Europe, although it has recently been used in Japan and half of the national production of honey comes from bees using this tree (Morimoto *et al*., 2010; Vítková *et al*., 2017a; Alilla *et al*., 2022).

1.3.6. Native range of *R. pseudoacia*

Robinia pseudoacacia is native to the Appalachian Mountains in North America in Pennsylvania and southern Ohio, eastern Alabama, northern Georgia, South Carolina and Alabama. Another population can be found in the Ozark Mountains of southern Missouri, northern Arkansas, and north-eastern Oklahoma (figure 1.10) (Huntley, 1990; Mally *et al*., 2021). It was commonly planted and has become naturalized all-over North America and southern Canada (Huntley, 1990). There it forms part of mixed mesophytic forests on slopes and forest edges (Boring and Swank, 1984; Rice *et al*., 2004; Vítková, 2015a). In these forests, at high elevations, it is found alongside trees including *Pinus echinata* Mill. (Pinaceae), *Pinus pungens* Lamb. (Pinaceae), *Quercus coccinea* L. (Fagaceae), *Pinus rigida* Mill. (Pinaceae) and *Kalmia latifolia* L. (Ericaceae) in variations of oak hickory forest (Boring *et al*., 1981). At mid-range elevations, forests are primarily governed by *Quercus prinus* L. (Fagaceae), *Quercus coccinea* L. (Fagaceae), *Quercus velutina*, L. (Fagaceae), and *Carya* spp. Nutt. (Juglandaceae). In addition to *R. pseudoacacia*, *Liriodendron tulipifera* L. (Magnoliaceae), *Tsuga canadensis* L. (Pinaceae), and *Acer rubrum* L. (Sapindaceae) are observed at lower altitudes and within ravine areas (Boring *et al*., 1981; Chang *et al*., 1998).

Robinia pseudoacacia is a strong pioneer plant because of its nitrogen fixing ability and root suckering. It follows disturbances and dominates secondary successional habitats in temperate regions of North America (Boring *et al*., 1981; Huntley, 1990; Chang *et al*., 1998).

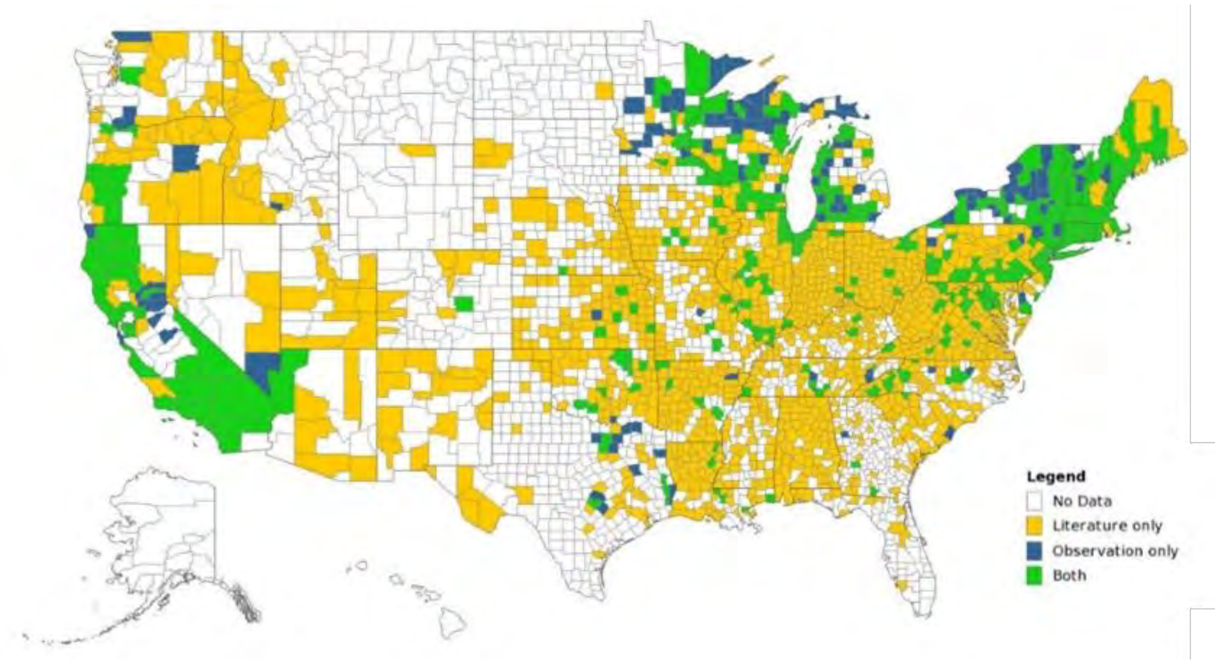

Figure 1.10. A map of *Robinia pseudoacacia* occurrences in its native range. Yellow indicates where *R. pseudoacacia* is reported in the literature. Blue indicates where it has been observed and green indicates an overlap of both (EDDMapS, 2019)

1.3.7. Invaded and introduced range of *R. pseudoacia*

Except for *Querus ruba* L. (Fabaceae), *R. pseudoacacia* has become the most common angiosperm introduced to Europe for wood production. It was first introduced to Europe in the beginning of the 17th century and was one of the first trees to be taken from North America (Huntley, 1990; Vítková & Kolbek, 2010; Vítková *et al*., 2015). Early records show that the first *R. pseudoacacia* plantation was in 1785 within the Czech Republic (Kadlec *et al*., 2018). It was seen as a popular plant because it had many uses, grew rapidly and was resistant to insects and fungi (Cierjacks *et al*., 2013). It was also introduced as a rare ornamental exotic plant to botanical gardens and quickly distributed all over Europe, with Paris as the most important distribution centre (Cierjacks *et al*., 2013). The arrival in Slovenia was the latest introduction of *R. pseudoacacia* in Central Europe in the beginning of the 19th century and was thought to have been brought over from northern Italy (Rudolf & Brus, 2006).

Later it became known as an invasive alien plant and is currently listed amongst the 40 most invasive woody angiosperms in the world. However, the beginning of the *R. pseudoacacia* invasion was not documented well and it is unclear why it had a late start (Vítková *et al*., 2017b). The first record of *R. pseudoacacia* escaping and becoming an invasive tree was in Germany in 1824, about 200 years after its first introduction to the continent (Vítková *et al*., 2017b). It's thought that the climate might have been cooler and less favourable which slowed its invasion but then accelerated due to the emergence of urban and industrial landscapes, making it more suitable for growth (Sukopp & Wurzel, 2003). It is reported as naturalized in 154 regions out of a total of 843 in the world (figure 1.11) (Van Kleunen *et al*., 2015). In several European databases *R. pseudoacacia* is classified as highly invasive (e.g. CABI, 2019; EPPO, 2002) and is included in national Black Lists and checklists of alien species across Europe (Celesti-Grapow *et al*., 2009; Pyšek *et al*., 2012; Pergl *et al*., 2016; Brus *et al*., 2019).

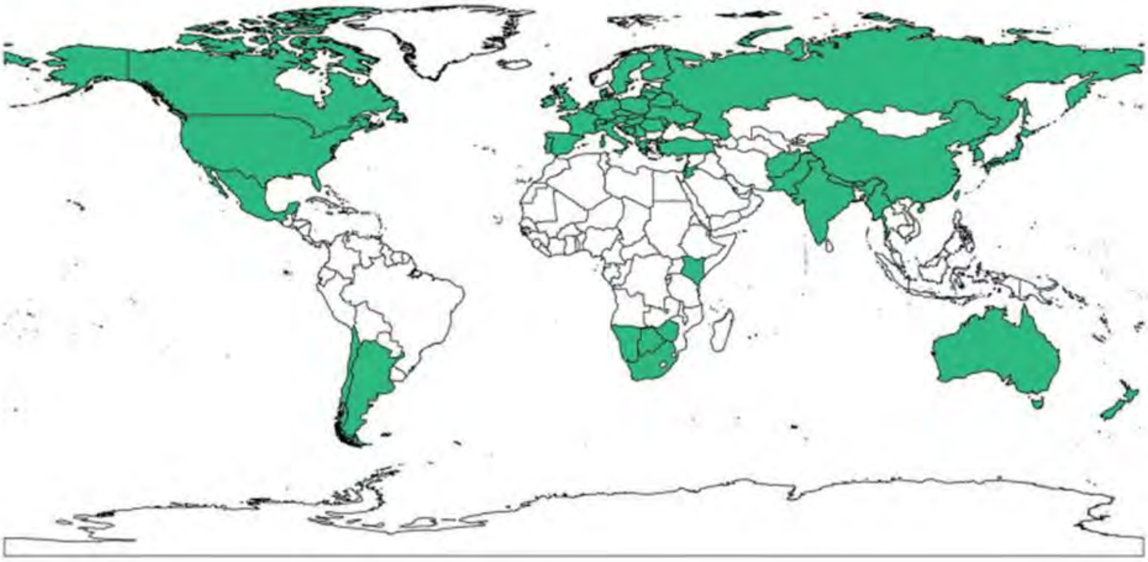

Figure 1.11. A map of *Robinia pseudoacacia* in its introduced range. Green indicates distribution based on published sources (Boer, 2013)

According to Cseresnyés & Csontos (2012), *R. pseudoacacia* has increased in abundance because it has escaped from parks, gardens and plantations where it had been purposely planted (figure 1.12). Plants were also escaping from cultivation and most likely spread clonally from these sites, but such escapes were only occasional and thus not given attention (Pergl *et al*., 2016). Although the potential for *R.*

pseudoacacia to become invasive was already apparent before World War II, the species was perceived as a commonly naturalized tree without any negative impact on native communities. After 1945, *R. pseudoacacia* specifically invaded the rubble left after bombing of urban sites where it occurred as a spontaneous pioneering tree in areas where climate was suitable (Vítková *et al*., 2017a). The steady increase of *R. pseudoacacia* stands have been recorded since 1950s (Berg *et al*., 2016). After it was considered invasive, stumps of harvested trees were found to resprout rapidly (Krízsik & Körmöczi, 2000) and eradication with the aim of restoring original plant communities was very difficult, costly and time-consuming (Ivajnšič *et al*., 2012; Skowronek *et al*., 2014).

In the South African context, *R. pseudoacacia* holds dual significance as both a valued silviculture species and a concerning invasive alien plant (Humphrey, 2016; Martin, 2019; Chikowore; 2021). The species has established a widespread presence across all nine provinces, with particularly abundant occurrences in Gauteng, Free State, Mpumalanga, and Eastern Cape Provinces (Humphrey, 2016; Martin, 2019). Notable infestations have been observed in various locations, including Clarens in the Free State Province, Pilgrims Rest in Mpumalanga Province, Majuba in KwaZulu-Natal Province, and between Barkley East and Rhodes in the Eastern Cape Province (Henderson, 2001; Martin, 2019). The recorded sites have shown a consistent increase over time, from 110 quarter degree squares (QDS) in 2000 to 159 QDS in 2015, indicating a progressive expansion of its distribution (Humphrey, 2016; Martin, 2019).

Robinia pseudoacacia's invasiveness is particularly pronounced in urban areas, along roadsides, watercourses, and grasslands, where it has often been intentionally planted for ground reclamation and soil stabilization in eroded gullies (Martin, 2019). The species demonstrates a notable potential for invasion, with a distribution map suggesting it could invade up to 46.3% of South Africa, albeit with the lowest probability in the western section, mainly in the Northern Cape Province, and certain areas in Limpopo Province and along the eastern coastline (Humphrey, 2016; Martin, 2019). This expanding distribution underscores the urgency of implementing effective management strategies in South Africa to mitigate the ecological impact of *R. pseudoacacia* in the Southern Hemisphere, where limited funding often challenges comprehensive control measures (Humphrey, 2016). The escalating spread of black locust necessitates careful consideration and the development of sustainable management approaches to balance its economic benefits in silviculture with the preservation of South Africa's native ecosystems.

Figure 1.12. *Robinia pseudoacacia* is known to escape from areas where it has been grown, for example in gardens as ornamental plants

1.3.8. Management of *R. pseudoacia*

In its native range *R. pseudoacacia* is susceptible to several herbivores and pathogens, for example, witches' broom disease caused by the virus *Chlorogenus robiniae,* and fungal diseases that cause heart rot. It is also vulnerable to damage from native insects such as the locust borer, *Megacyllene robiniae* Forster (Cerambycidae), and the locust leafminer *Odontota dorsalis* Thunberg (Chrysomelidae), (Michigan Department of Natural Resources, 2012). Insects found on *R. pseudoacacia* in its native range are well studied and there is high potential to identify a successful control agent to reduce the spread and potentially the density of this invasive tree (Sheppard *et al*., 2006).

In South Africa it has become important to find a management strategy for *R. pseudoacacia* as it is spreading quickly and is already impacting the agricultural sector negatively (Humphrey *et al*., 2019). It will be important to plan a control program. Resources for invasive species control often fall short of the actual need, so it is important to prioritize sites for treatment. Assessing both the scope of the problem and any available resources is the first step (Michigan Department of Natural Resources Michigan, 2012). According to the Michigan Department of Natural Resources (2012) there are several points to consider in developing a strategy for control. It is important to map known populations and observe whether they consist of scattered or single specimens and if extensive clones are present and if the spread is clonal or by single plants. It will also be necessary to take into account if these populations are on high value sites, natural areas or sites of cultural value. Whether the stands are sparsely scattered in otherwise native vegetation or whether they cover large areas of low-quality habitat will affect control efforts and high value sites should then be prioritized. Appropriate control methods should be considered carefully, given the growth habit of the species, site conditions and available resources (Michigan Department of Natural Resources, 2012; Cierjacks *et al*., 2013; Humphrey *et al*., 2019). The potential for harm to non-target species is also important when considering control options and adequate provision to minimize damage to native flora should be made. Understanding the species is vital as clones are more problematic because the entire clone should be treated, while smaller satellite populations can be eradicated (Cierjacks *et al*., 2013; Humphrey *et al*., 2019). Populations that have been treated should then be consistently monitored to ensure that cut and treated stumps do not resprout.

If a single stem survives, the entire clone may regenerate (Michigan Department of Natural Resources, 2012; Cierjacks *et al*., 2013; Humphrey *et al*., 2019).

In the past, control of established *R. pseudoacacia* populations have required the use of herbicides, although mechanical control may occasionally have enhanced the effectiveness of other methods (DeLoach, 1997; Stone, 2009; Michigan Department of Natural Resources, 2012). Mechanical control involves practices such as hand-pulling, digging or bull-dozing, cutting or mowing and grazing. Since most *R. pseudoacacia* spread is vegetative, and saplings are connected underground, hand-pulling is not useful. Cutting trees and removing the roots with a bulldozer has been suggested but does not appear practical or cost efficient in most cases (Henderson, 2001). Cutting without the use of herbicide produces prolific sprouts and should not be advised. Between cut and uncut sites, those that had been cut had twice the growth rates of those that had not been cut, and began to flower in half the time (Brown *et al*., 2001; Edgin, 2007; Humphrey *et al*., 2019). According to the Michigan Department of Natural Resources (2012) browsing by a combination of cattle and domestic goats effectively controlled the height of *R. pseudoacacia*. After 4 seasons, all trees had died but *R. pseudoacacia* can be toxic to cattle and other animals and caution should be advised. It is however grown specifically to feed domestic goats in Pakistan and New Zealand, and they appear to be a better choice for control in terms of grazing (Burner *et al*., 2008).

As mentioned, to effectively control *R. pseudoacacia* the use of herbicides is still usually required. Nevertheless, it can negatively impact natural areas such as wetlands, water sources, native vegetation and areas prone to erosion (Michigan Department of Natural Resources Michigan, 2012). Triclopyr provides effective control of broad-leaved plants but does not kill grasses and some conifers. It is used as a foliar spray, although spraying is generally used to control resprouts following other methods such as mechanical cutting (Michigan Department of Natural Resources, 2012; Martin, 2019) . Triclopyr can be used in combination with imazapyr which acts over an extended period of time. Because it can persist in the soil, it provides improved control but can also kill valuable non-target species (Michigan Department of Natural Resources Michigan, 2012). Triclopyr can also be used in combination with aminopyralid, which is lethal to legumes - an advantage in the case of *R. pseudoacacia*. Aminopyralid is particularly useful in providing effective control in grassland habitats. It also remains active in the soil for long periods of time and should not be used around other trees (Michigan Department of Natural Resources Michigan, 2012).

Foliar application of herbicide can be beneficial at sites with widespread *R. pseudoacacia* populations and few desirable native plant species. The herbicide should be applied after the leaves are fully expanded to actively growing plants, although during periods of drought or other stress, it may not be effective (Tu *et al*., 2001). Cut-stump treatment may be used in any season except during periods of heavy sap flow in spring. It is useful for species like *R. pseudoacacia* that normally resprout after cutting. After the stems have been cut, they are painted with concentrated herbicide, using a spray bottle or wicking applicator (Tu *et al*., 2001; Stone, 2009). Basal bark treatment can be used on stems less than six inches in diameter at any time of year except during heavy sap flow in spring. It is most useful during the dormant season. In basal bark treatment, concentrated herbicide is applied to a band of bark around *R. pseudoacacia* stems extending up 30 cm from the ground. Basal bark treatment is most effective on younger stems with thin bark (Tu *et al*., 2001; Michigan Department of Natural Resources, 2012). Drill and fill, and injection techniques are useful on larger trees. They leave the tree in place to break down over time, providing valuable habitat and structure at the same time. They can be used any time of year except during spring sap flow. The drill and fill technique involves drilling holes into the tree at a downward angle and filling them with a measured amount of concentrated herbicide using a squirt bottle (Tu *et al*., 2001; Michigan Department of Natural Resources, 2012). In fire-adapted communities, prescribed burning may enhance control of *R. pseudoacacia* over the long term, but should always be considered as part of an integrated management plan for the site as it will stimulate the species over shorter time spans. When prescribed burning is initiated, it should be supplemented with other control methods (Stone, 2009; Michigan Department of Natural Resources, 2012).

Herbicidal control has not always been successful, and other solutions should be considered (Brown *et al*., 2001; Czarapata, 2005; Edgin, 2007; Cierjacks *et al*., 2013). The next step in managing *R. pseudoacacia* would be to incorporate biological control. It is important then to prioritise which biological control agent will be used to manage *R. pseudoacacia*. According to literature, seed-feeding insects should be investigated first as they are the most cost-effective option and are able to sustain themselves in the long term (Richardson & Kluge, 2008; Van van Wilgen *et al*., 2012). It was found that there was an 80 % decline in *A.saligna* seedbank after a time period between four to six years, a tree belonging to the Fabaceae family as well (Holmes & Rebelo, 1988).

1.4. Aims and Structure

The aim of this study is to develop and potentially release biological control agents for the management of *R. pseudoacacia* in South Africa. With the use of available resources, scoring systems, citizen science initiatives, ecological niche modelling and pre-release survey data potential biological control agents can be identified. Using literature and phylogenetics the safety of these selected biological control agents can then be estimated for release.

Chapter 1, examines biological control as an alternative to mechanical and chemical methods, focusing on safety and applicability in South Africa. It provides insights into the target weed, its origin, use, and impact, emphasizing the importance of understanding the release area before initiating a biological control program.

Chapter 2, conducts a pre-release study to assess the invasive tree's impact and interactions in selected study sites. It uses information from Chapter 1 to inform the choice of a leaf-feeding species for biological control.

Chapter 3, uses findings from the pre-release study and available literature to identify potential leaf-feeding species with high efficacy. The results build upon the data from Chapter 2. With the decision to use a leaf-feeding insect established in Chapter 3,

Chapter 4, uses climatic variables to filter and identify the most suitable candidate for South Africa. The ecological niche models in this chapter build upon insights from earlier chapters.

Chapter 5, replicates a scenario where a biological control agent was introduced without specificity testing, drawing inspiration from Chapter 4, where climatic variables were used to assess compatibility.

Chapter 6, integrates the findings and implications of the preceding chapters, emphasizing the integration of information and insights. The structured progression contributes to a comprehensive understanding of using a leaf-feeding biological control agent against *R. pseudoacacia* in South Africa.

Chapter 2. Pre-release Surveys of *Robinia pseudoacacia* in South Africa

2.1. Introduction

After habitat destruction, biological invasions are considered the greatest threat to the integrity of native ecosystems and biodiversity (Wilcove *et al*., 1998; Richardson & Van Wilgen, 2004). An invasive alien species is defined as an alien species that acts as an agent of change and is a threat to indigenous biodiversity or causes economic harm when it becomes established in areas outside of its native range (Sabo, 2000; Richardson & Van Wilgen, 2004; Reid *et al*., 2009). The invasive plants outcompete and displace native species for resources resulting in a complete alteration of ecosystem dynamics, thereby decreasing biodiversity and negatively impacting conservation efforts (Reid *et al*., 2009; Paterson *et al*., 2011; Van Wilgen *et al*., 2012). Not only do the negative impacts of invasive aliens affect vertebrates, invertebrates and native plants but also soil microbes, disturbance regimes (fire), food webs, ecosystem processes (nutrient cycles), soil compositions and especially humans (Richardson & Van Wilgen, 2004; Reid *et al*., 2009). The three main consequences of invasive aliens are: biological; the eradication of natural capital, economic; the threat to economic productivity and ecological; jeopardising the stability of ecosystems (Richardson & Van Wilgen, 2004).

Robinia pseudoacacia is an early successional species that has a competitive advantage by rapid spread and is thought to be the second most common deciduous tree worldwide (Akamatsu *et al*., 2011). It is considered highly invasive, classified as a category 1B invasive alien plant in South Africa, although it has economic and ecological value (Martin, 2019). Category 1B invasive species (National Environmental Management: Biodiversity Act 2004) require mandatory control in the form of removal and destruction. This control must be part of an invasive species control programme (Martin, 2019). The negative impacts of *R. pseudoacacia* invasions are mostly associated with the decrease of biodiversity, especially native grasses as *R. pseudoacacia* stands alter the amount of shade in invaded areas and change soil compositions (Cierjacks *et al*., 2013). Some of the economic values of *R.*

pseudoacacia include its use in the timber industry, apiculture, and for soil stability in disturbed areas. It is also used as a source of nectar for honey and shade in metropolitan areas (Cierjacks *et al*., 2013; Masaka *et al*., 2010; Martin, 2019).

In South Africa, the only available control methods for *R. pseudoacacia* at the moment are a combination of mechanical and chemical techniques (Martin, 2019). This involves the incomplete girdling of trees after which an herbicide is applied. This method appears to prevent both basal regrowth and continued growth of the tree (Sabo, 2000; Cierjacks *et al*., 2013). While this may prove to assist in controlling the tree, other methods of control must be studied to formulate the most efficient IMP. A possible solution may be biological control (Cierjacks *et al*., 2013).

The first step to starting a biological control program is to ensure that there are no potential agents present in the introduced range. For example in 1982, four weevil species were imported into the quarantine facility for the control of *Sesbania punicea* (Fabaceae) (Hoffmann & Moran, 1991). Two years later however it was found that one potential agent, *Trichapion lativentre* (Curculionidae), had already become established in South Africa and was widely distributed (Hoffmann & Moran, 1991). In some cases, insects associated with invasive alien plants escape with their host into the invaded range. This is the case with *Schinus terebinthifolia* (Anacardiaceae) another invasive alien in South Africa as well as North America. Pre-release surveys done in Florida showed the presence of a seed-feeding wasp, *Megastigmus transvaalensis* (Hymenoptera: Torymidae), that had been unintentionally introduced into the country. In North America it is now regarded as an important part of the control of the weed as it's reported to reduce seed capacity up to 76 % (Wheeler *et al*., 2001). The seed-feeding wasp is however native to South Africa where it is associated with the *Rhus* genus, while the weed is native to South America (Scheffer & Grissell, 2003). This is not typical of usual biological control agents as the geographic and host range is much larger than what is preferred but if the country has no other native or economically important plants of the same genus, it can be allowed. Within the distribution range of the weed in North America, it was found that no other members of the Anacardiaceae, native to the area or economically important, were attacked by the wasp, including results from host-specificity tests (Wheeler *et al*., 2001). It is therefore considered a successful biological control agent in North America.

To ascertain the effectiveness of a biological control agent it is important to document the invasive plant and its negative impacts before releasing it (Müller-Schärer & Schaffner, 2008). Pre-release studies, as well as post-release studies, are important for the overall improvement of biological control and should be included in biological control programmes (McFadyen, 1988). Pre-release studies provide information on the ecology of different invasive populations, ensuring that potential agents with the highest likelihood of success are selected to import into quarantine. It also provides baseline data on which post-release success can be measured. Without it, evaluating success may become inconclusive (McFadyen, 1998; Blossey *et al*., 2018). These evaluations provide justification for funders and regulatory agencies to continue investing in biological control efforts (McFadyen, 1998; Blossey *et al*., 2018). Another advantage to pre-release studies is that it gives insight into different invasive populations in the same invaded range. This is important when considering invasive plants that have different reproduction cycles within the invaded range compared to their native range (Edwards, 1998). For example, Barker *et al*., (2016) explains that invasive populations of *Centaurea solstitialis* flower earlier, produce larger seeds and grow larger in the invasive range than in their native range. Another example from Coetzee *et al*. (2021) shows that both *Egeria densa* and *Myriophyllum aquaticum* are dioecious aquatic plants capable of both sexual and asexual reproduction, but in South Africa, where they are invasive, only female plants can be found and therefore the plant relies solely on asexual reproduction. This type of pre-release data saves valuable time and money when invasive populations are thoroughly understood. De Clerck-Floate & Bourchier (2000) state that "Linking population ecology with biocontrol can essentially help to select more appropriate control agents; increase the likelihood of agent establishment; and support greater predictability of the interactions between the host and control agent populations". Population ecology will therefore benefit biological control programmes in a more general sense, whereas population dynamics may help biological control programmes in more targeted scenarios.

It is therefore vital to study the invasive plant's population dynamics in order to set goals and pre-determined thresholds in the invaded range, which will then be an indication of the levels of control needed and how successful that agent has been (Bourchier *et al*., 2006; Müller-Schärer & Schaffner, 2008; Paterson *et al.*, 2011). Understanding the population dynamics of the weed before implementation of the biological control programme will be beneficial to determining whether it was successful or not as biological control does not eradicate the weed and the effects of biological control also take longer to surface than mechanical or chemical control (Bourchier *et al.*, 2006; Paterson *et al.*, 2011).

Thus, the aims of this study were to determine whether the insects associated with *R. pseudoacacia* are specifically phytophagous, and in addition to determine what guilds should be targeted for biological control and to collect valuable pre-release data useful for post- release evaluations.

2.2. Materials and Methods

2.2.1. Site Selection

Robinia pseudoacacia was first recorded in South Africa in 1895 in the Eastern Cape Province in the Conservator of Forests report by Sim (1907) (Martin, 2019). It has since spread throughout the country with large populations in the Free State, Gauteng, Mpumalanga and Eastern Cape Province (Figure 2.1) (Martin, 2019).

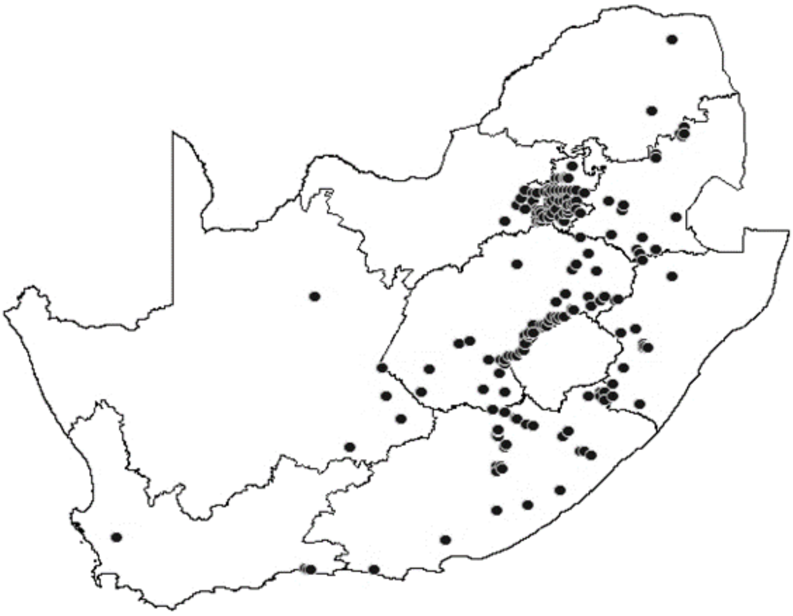

Figure 2.1. The distribution of *Robinia pseudoacacia* in South Africa (Martin, 2019)

In total 23 sites in Mpumalanga, North West, Free State and Eastern Cape were selected. For the pre-release survey, different aspects were observed for all *R. pseudoacacia* stands within these sites. These included determining which insects were found on the trees, assessing the amount of damage observable on the leaves, recording the amount of seeds still on the trees within the pods, the amount that has fallen and forms the seedbank and measuring population densities. Appendix 2.1 indicates the name of the site (either the adjacent town or farm, nearby landmarks or surrounding suburb(s)), and the shortened code for that site and its coordinates.

2.2.2. Insect Surveys

Insect surveys were conducted at all the sites within the Free State. These include SunnySide 1, SunnySide 2, SunnySide 3, Clifton 1, Clifton 2, Clifton 3, Clarens, BVHS, Jordania, BHM Dump and Panorama. Each survey was conducted between February and March to remain within the same season.

At each site, the first step was to actively search for insects on the trees. A few minutes were spent, by a minimum of two researchers, getting accustomed to the study sites followed by a five-minute active search.

(In my next life I want to be a plant ecologist!)

2.2.3. Leaf Damage Surveys

To quantify leaf damage all 21 sites were surveyed. At each site 30 leaves (approximately 15 to 20 leaflets) from 5 different trees and at varying heights were randomly assessed. The leaves were categorized into a range of percentages according to the damage on each leaf. This was done by each person estimating the percentage damage. The damage of each leaflet was then totalled to account for one leaf. Table 2.1 explains the categories in which the leaves were placed according to percentage damage from a range. The type of insect damage was also considered. This was based on cutting, chewing, rolling, and mining damage.

Table 2.1. The categories used to determine which percentage range leaf damage was present.

Category	Percentage range (%)
A	0
B	0 - 5
C	6 - 10
D	11 - 20
E	21 - 50
F	51 - 100

The average height of the trees within the stand was measured, and the type of stand was determined according to another set of categories (table 2.2). It was noted whether stands/infestations were disturbed, i.e., there was evidence of mechanical removing or branches being chopped off, or undisturbed, i.e., the trees have been left alone and grow unencumbered by human disturbance.

Table 2.2. The type of *Robinia pseudoacacia* stand according to set categories and tree species

Category	Stand type
A	Only *R. pseudoacacia* present in the stand in a rural area
B	*R. pseudoacacia* and other tree species in a rural area

C	Only *R. pseudoacacia* in an urban area
D	*R. pseudoacacia* and other tree species in an urban area

2.2.4. Seed Surveys

Seedpods present on the trees were collected at all the sites where they were present on the tree (figure 2.2.). At sites where there were more than 50 pods, only 50 pods were collected. They were stored in brown paper bags according to the different sites where they were collected to take back to the laboratory. This was done to establish the number of damaged seeds within the pods.

Figure 2.2. Seed pods still present on *Robinia pseudoacacia* that were collected.

At each site seed rain traps were also set up (figure 2.3). They consisted of a piece of PVC pipe with half a 2 L bottle inside. The nozzle of the bottle had a piece of gauze placed in the inside to allow water to drain but prevent seeds from washing away. The pipe was then kept in place by attaching two metal droppers on either side with cable ties and hammering them into the ground directly below the canopy.

Figure 2.3. The seed rain traps mounted below trees to collect seeds falling from *Robinia pseudoacacia.*

2.2.5. Seedbank Surveys

Seedbank surveys were conducted at 11 sites, namely SunnySide 1, SunnySide 2, SunnySide 3, Clifton 1, Clifton 2, Clifton 3, Clarens, BVHS, Jordania, BHM Dump and Panorama. To establish the amount of seed in the seed bank a soil augur was used to remove a section in the top layer of soil (figure 2.4). The augur removes 20 x 5 cm of soil in a cylinder. The soil was placed into small plastic bags and labelled according to each site to take back to the laboratory. The formula to establish the amount of soil removed by the augur is: $V = \pi r^2 h$. In the lab a sieve with a grid of 1 x 1 mm was used to filter the soil and seeds were counted and collected.

Figure 2.4. A soil augur was used to take a sample from the top layer of soil. A sieve was used to sieve soil samples to establish how much seed was present in the seed bank.

2.2.6. Camera traps

To determine the mechanisms of spread dispersal, trap cameras (Bushnell Trophy Cam HD Max-Colour LCD, Bushnell Outdoor Products, Overland Park, KS, USA) were used by following methods from Zoeller *et al*. (2016). Motion sensors picked up movement triggering 60 second videos at 10 second intervals. Bait stations were placed in front of the cameras with *R. pseudoacacia* seeds, at some of the stations *Pyracantha angustifolia* berries or *Rosa rubignosa* berries could be seen. Six cameras were placed at the study sites for five days during July 2021 and then again in March 2022. Cameras were placed 25 cm away from the bait stations. After the cameras were retrieved, birds and mammals were identified using Sinclair & Ryan (2009) and Stuart & Stuart (2015) from the video footage.

2.2.7. Population Density Surveys of *R. pseudoacacia*

Density surveys were conducted at 7 sites, namely SunnySide 1, SunnySide 2, SunnySide 3, Clifton 1, Clifton 2, Clifton 3 and Clarens. At each site quadrats were used to establish population density. Generally, larger quadrats are better than smaller ones and they should always be larger than the largest growth forms (Kent, 2012).

Sites were categorised as disturbed or undisturbed depending on whether there had been cutting of branches or chopping trees down - most likely for firewood. Sites categorised as undisturbed were SunnySide 1, SunnySide 2, SunnySide 3 and Clifton 1. Sites categorised as disturbed were SunnySide 4, Clifton 2, Clifton 3 and Clarens.

A tape measure was used to measure 10 x 10 m quadrats within each site, as suggested for woodland shrubs and small trees, and was repeated at least five times for each site. The number of individual trees was then counted within each quadrat, and the DDBH (Diameter at Breast Height) of each tree was measured. At sites where *R. pseudoacacia* had been disturbed, smaller quadrats, measuring 5 x 5 m, were used instead as the shape of the tree changes to a shrub and becomes a dense thicket that proves difficult to move through.

2.2.8. Statistical analysis

GraphPad Prism 5 was used for statistical analysis. Significance was determined by a P value smaller than 0.05 ($P < 0.05$). The D'Agostino & Pearson omnibus test was used to determine normality of the data and the appropriate additional statistical analyses were used. The Mann-Whitney test was used for nonparametric data, which does not assume a normal distribution. It identifies if one of two random variables are stochastically larger than the other. Multiple t-tests were used to determine whether the means between more than two parametric sets of data were significantly different from each other. For rank- based nonparametric tests the Kruskal–Wallis test (ANOVAs) were used to determine statistically significant differences between two or more groups of an independent variable on a constant dependant variable.

2.3. Results

2.3.1. Insect Surveys

Total survey time for 5 min active searches at 7 sites over 3 years with 3 extra sites for 2020 (lockdown) was 3 hours and 15 min Table 2.3.).

Table 2.3. Insects found on *Robinia pseudoacacia* during five-minute active searches at each site. Individuals (n) from different orders noted

Order	Clarens		Clifton 1		Clifton 2		Clifton 3		Sunnyside 1		Sunnyside 2		Sunnyside 3	
	n	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*
Hemiptera	9	31.0	1	4.0	12	44.4	7	43.8	9	42.9	17	32.7	15	45.5
Orthoptera	1	3.4	0	0.0	1	3.7	1	6.3	0	0.0	1	1.9	1	3.0
Coleoptera	0	0.0	3	12.0	1	3.7	2	12.5	4	19.0	3	5.8	3	9.1
Diptera	16	55.2	19	76.0	11	40.7	6	37.5	7	33.3	22	42.3	13	39.4
Hymenoptera	1	3.4	2	8.0	0	0.0	0	0.0	0	0.0	9	17.3	0	0.0
Mantodea	0	0.0	0	0.0	0	0.0	0	0.0	1	4.8	0	0.0	0	0.0
Lepidoptera	2	6.9	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	3.0

Total	29	100	25	100	27	100	16	100	21	100	52	100	33	100

2.3.2. Leaf Damage Surveys

The sites visited from 2019 to 2022 (except during 2020 due to national lockdown regulations) were selected in the mountainous grassland of the eastern Free State. These consisted of one site in Clarens, three sites on Clifton farm and three sites on Sunnyside farm. Leaf damage was compared between these sites (figure 2.5.) and from an ANOVA test no significant difference ($P > 0.05$) was found in this dataset as well as from multiple comparisons being done.

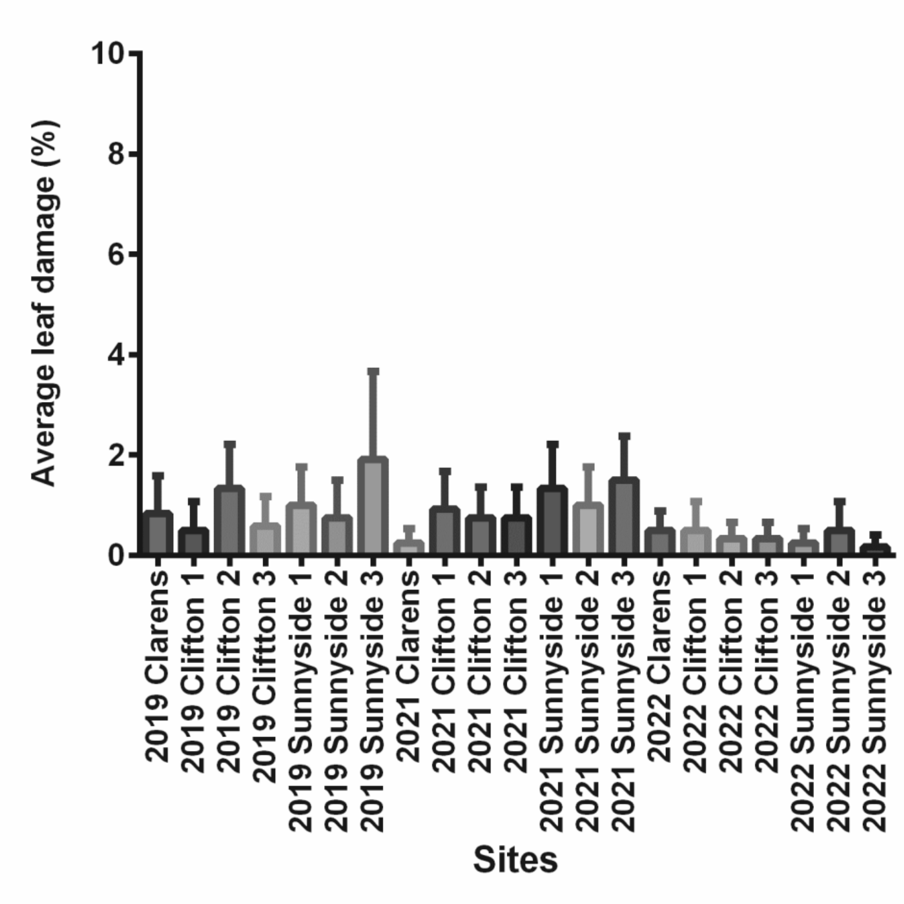

Figure 2.5. The average percentage of leaf damage from each site from 2019 to 2022. Note there are no data for 2020 due to national lockdown regulations.

Across all 21 sites the only insect damage that was observered was by insect chewing (cutting). A one-way ANOVA test on the percentage leaf damage (figure 2.6.) indicated statistical significance (P value < 0.5) by Dunn's multiple compassion test between the means of the 21 sites (615 values). From 210 comparisons 6 sites differed significantly with another site resulting in 28.5 % of sites that had some significance.

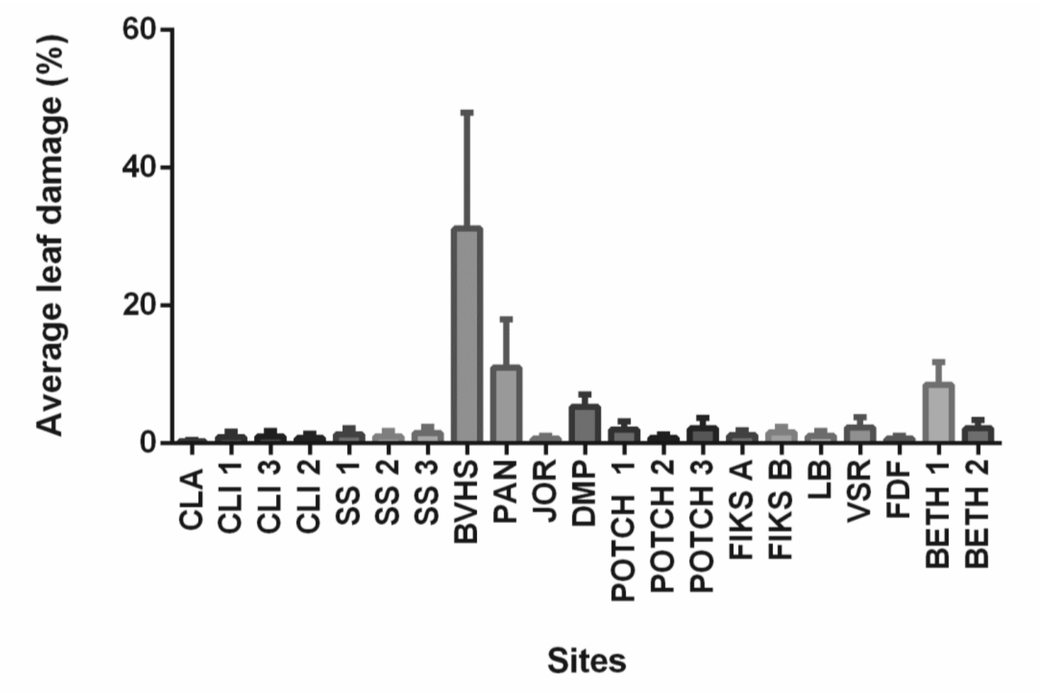

Figure 2.6. The average percentage of leaf damage across 21 sites from 4 provinces including Mpumalanga, North West, Free State and Eastern Cape. The only insect damage present was by cutting. Some of the sites differ significantly from one another.

To understand why there were differences in leaf damage between these sites, the stand types were compared between all the sites. A one-way ANOVA test was done on the stand types from all the sites (figure 2.7.). There was significance between the means ($P < 0.05$) of the 4 groups. Type A had less damage and was significantly different from Type D with an adjusted P value of < 0.0001. Type B was also significantly different from Type D with an adjusted P value of < 0.0001 as it was significantly lower as well and Type C was significantly different from Type D with an adjusted P value of < 0.0001 while also having less damage than type D.

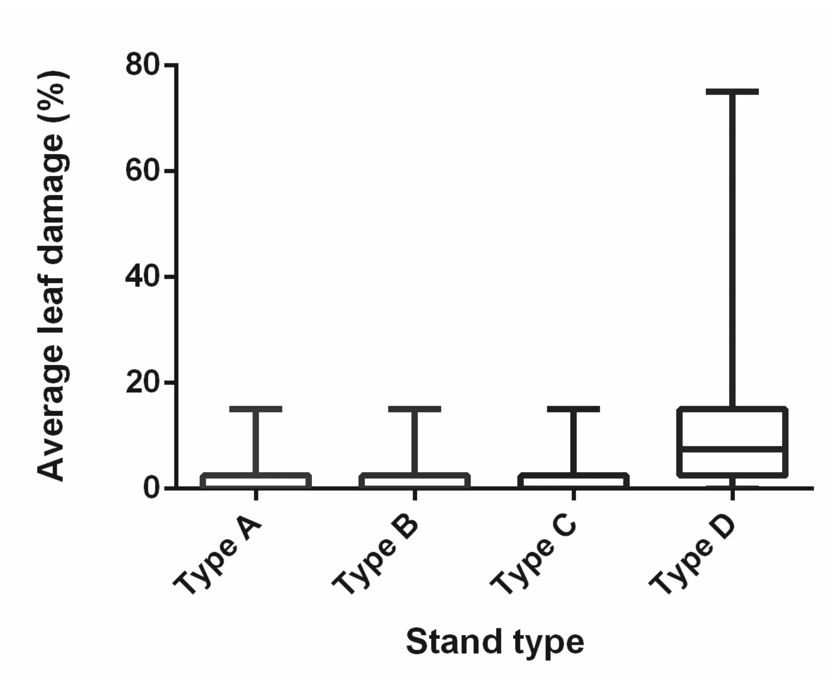

Figure 2.7. The average leaf damage in percentage compared to the different *Robinia pseudoacacia* stand types. Type A consisted of a monoculture of *R. pseudoacacia* trees in a rural environment. Type B consisted of mixed stands of *R. pseudoacacia* and other trees in a rural area. Type C consisted of a monoculture of *R. pseudoacacia* trees in an urban area and Type D consisted of mixed stands of *R. pseudoacacia* and other trees in an urban environment

To understand the extent of damage in the invaded range (South Africa), all the sampled sites during the study were compared to a single survey of leaf damage surveyed in 2020 in the native range of *R. pseudoacacia* in North America. Figure 2.8.a shows the average leaf damage total across all sites in South Africa and North America. A Mann-Whitney, two-tailed test shows that there is significant difference (P value of 0.0014) between South Africa and North America in terms of leaf damage. A closer look at the different sites within South Africa compared to the sites in North America (Figure 2.8.b.) reveals that all the Clarens sites in South Africa are significantly different from all the sites in North America. All the Clifton sites are significantly different from all the sites in North America too. Sunnyside 1 is significantly different from all the sites in North America except USA Riparian. Sunnyside 2 is significantly different from all the sites in North America and Sunnyside 3 is significantly different from all the sites in North America except USA Riparian. The sites situated in Bethlehem (type D sites) are not significantly different from the sites in North America. Except that site JOR in Bethlehem is significantly different from all the sites in North America, site DMP is significantly different from site USA Q and BETH2 is significantly different from site USA Riparian. Site POTCH 1 is significantly different from all the sites in North America except USA Riparian. POTCH 2 is significantly different from all the sites in North America and POTCH 3 is again significantly different from all the sites in North America except USA Riparian. Site FIK A is significantly different from all the sites in North America and FIK B is significantly different from all the sites in North America except USA Riparian. Site LB is significantly different from all the sites in North America, site VSR is significantly different from all the sites in North America except USA Riparian and site FDF is significantly different from all the sites in North America. Within North America, site USA progress is significantly different from site USA Q and site USA Q is significantly different from site USA Riparian.

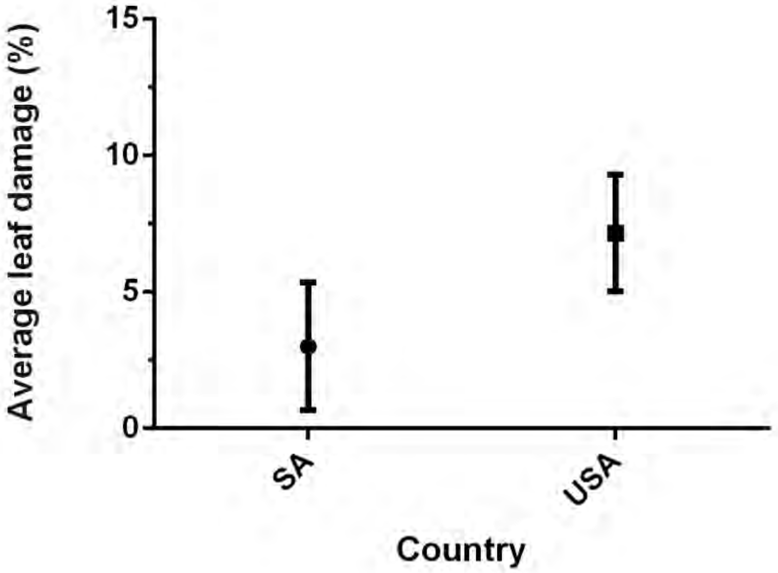

Figure 2.8.a. The total (means and standard deviations) of all the sites surveyed for leaf damage averaged in South Africa compared to a snapshot of surveys conducted in North America for leaf damage averaged in 2020.

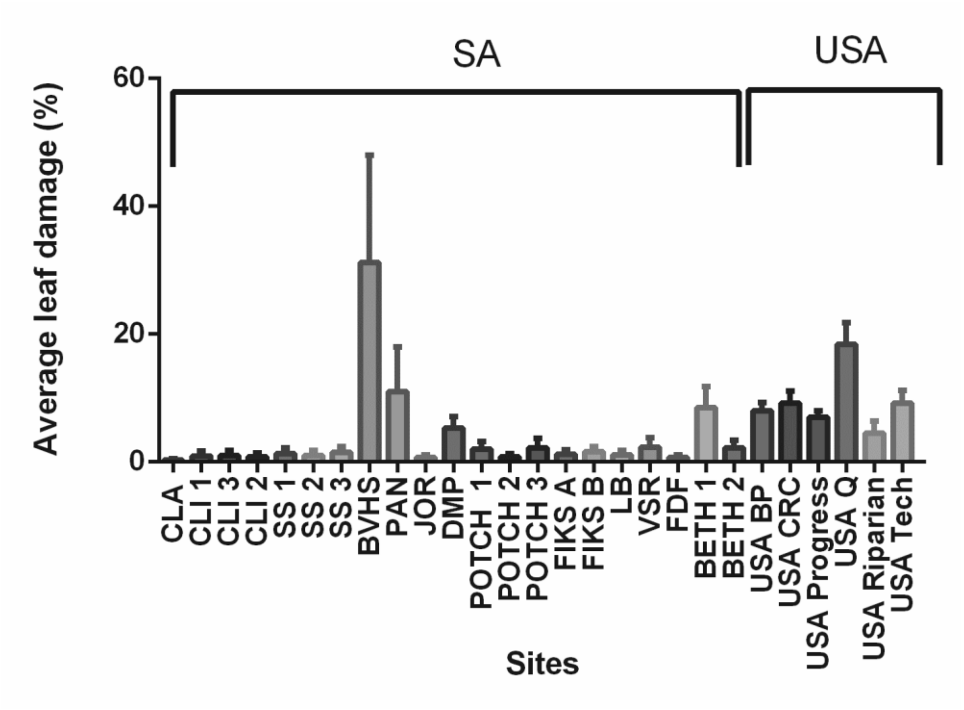

Figure 2.8.b. Sites (means and standard error) surveyed in South Africa for leaf damage averaged per site compared to sites surveyed in North America for leaf damage averaged per site.

Type of leaf damage was also assessed during leaf damage surveys (figure 2.9.). The types of damage observed in North America included marginal damage (damage to the leaf margins), cutting (chewing), leaf mining, leaf rolling, white window damage (top and bottom). The only damage observed in South Africa was cutting (chewing) damage. From a two-way ANOVA there is significant difference (P value of 0.0001) between types of damage when South Africa is compared to North America.

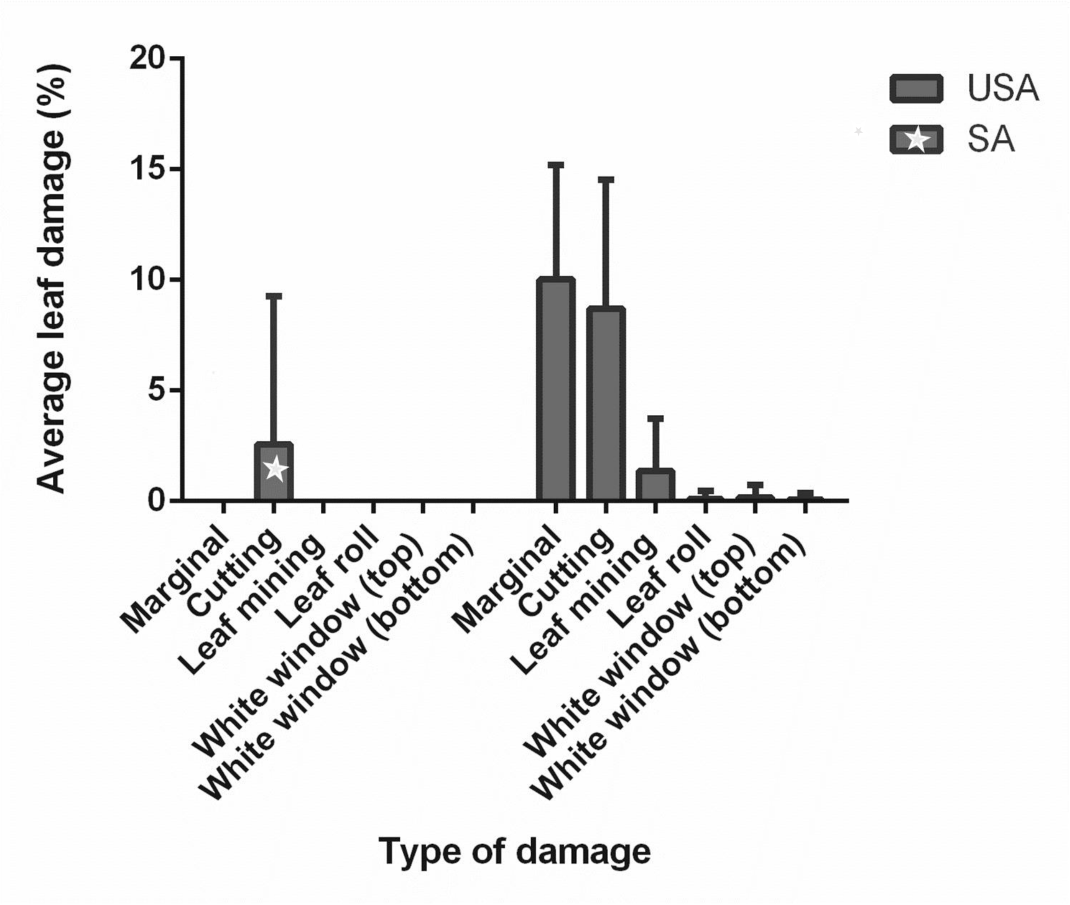

Figure 2.9. Type of damage observed on leaves of *Robinia pseudoacacia* during leaf damage surveys in South Africa and North America.

2.3.3. Seed Surveys

Seed pods were collected at sites where they were still present on the trees. Not all the sites had seed pods even though the pods stay on the trees year- round. Seeds were then carefully opened and the number of seeds were counted that were still inside and viable. The number of seeds that the pod could contain along with seeds damaged by insects were also counted. The percentage of viable seeds was then calculated and tabulated in table 2.4.

Table 2.4. The percentage viable seeds within seedpods collected at each site. Seeds missing or damaged by insects were noted to obtain the value.

Site	Percentage viable seeds (%)
Clifton 1 (2019)	43
Clifton 2 (2019)	76
Clarens (2019)	40
Bvhs (2020)	98
Sunnyside 1 (2021)	80
Sunnyside 2 (2021)	100
Clifton 1 (2021)	99
Clifton 2 (2021)	78
Clifton 3 (2021)	100
Bvhs (2021)	87
Sunnyside 1 (2022)	63
Clifton 3 (2022)	74

2.3.4. Seedbank Surveys

Overall the seedbank was very low. Over 3 years, in a total of 75 soil cores taken, only 15.8 % of cores taken in *R. pseudoacacia* stands contained *R. pseudoacacia* seeds. In total only 19 seeds were found at an average density of $6.6\text{x}10^{-4} \pm 2.1\text{x}10^{-3}$ seeds/cm^3.

2.3.5. Camera traps

Footage from the trap cameras caught a number of birds and mammals interested in the bait stations. Table 2.5. summarises the number of animals seen and whether or not they were interested in the *R. pseudoacacia* seeds.

Table 2.5. Birds and mammals observed from footage by camera traps and what interactions they had with the bait station composed of *Robinia pseudoacacia* seeds and sometimes *Pyracantha angustifolia* and *Rosa rubignosa* berries

July 2021			
Scientific name	**Common name**	**Number of observations**	**Observation**
Numida meleagris	Helmeted guinea fowl	1	Passing by
Sylvicapra grimmia	Common duiker	2	Eating *P. angustifolia* berries nearby
Streptopelia capicola	Cape turtle dove	1	Passing by
Cossypha caffra	Cape robin-chat	14	Passing by
Rhabdomys pumilio	Four-striped mouse	1	Scavenging, possibly feeding on *R. pseudoaccia* seeds
Euplectes capensis	Yellow bishop (non-breeding)	1	Scavenging, possibly feeding on *R. pseudoaccia* seeds

Hystrix spp.	Cape porcupine	1	Passing by
Trachyphonus vaillantii	Crested barbet	1	Passing by

March 2022

Scientific name	**Common name**	**Number of observations**	**Observation**
Sylvicapra grimmia	Common duiker	1	Eating *P. angustifolia* berries nearby
Upupa africana	African hoopoe	1	Passing by
Cossypha caffra	Cape robin-chat	3	Passing by
Rhabdomys pumilio	Four-striped mouse	6	Scavenging and eating nearby *R. rubignosa* berry and Clearly feeding on *R. pseudoacacia* seeds
Papio ursinus	Cape baboon	1	Passing by
Emberiza capensis	Cape bunting	1	Scavaging

The bait stations were mostly visited by Cape robin-chats but they didn't seem interested in the bait stations but rather curious about the cameras and the recent disturbance. Furthermore, according to Sinclair & Ryan (2009) Cape robin-chats' diets consist mainly of insects and other invertebrates. Sometimes they feed on fruit, kitchen scraps and small vertebrates. Therefore it is no surprise that they are not interested in the seed bait station. A crested barbet was also captured scavenging but their diet also does not consist of seeds (Sinclair & Ryan, 2009).

A yellow bishop was captured once by the cameara traps and was seen scavenging among the *R. pseudoacacia* seeds, but there was no clear picture of feeding. It is possible that it would eat *R. pseudoacacia* seeds as their diet consists of seeds and insects (Sinclair & Ryan, 2009). A Cape bunting (figure 2.10) was also captured once scavenging between the seeds. It was not clear from the footage if anything was fed upon although it would be quite likely that it would as their diets consist of seeds, fruit, buds and insects.

Several duiker and baboons were captured passing by, occasionally the camera traps would pick up footage of duiker feeding on the *P. angustifolia* berries nearby but they were not interested in the *R. pseudoacacia* seed. However, after examining eland droppings at one of the sites on a whim, 46 *R. pseudoacacia* seeds were found that had passed through the gut.

The four-striped mouse (figure 2.10) was a regular at most of the bait stations that had some grass cover around them. There was also clear footage of the mice feeding on *R. pseudoacacia* seeds as well as *R. rubignosa* berries that were nearby. According to Stuart & Stuart (2015) their main diet consists of seeds but they will also feed on other plant parts and insects.

Figure 2.10. Video snapshots of four-striped mice and a Cape bunting captured by camera traps scavenging among *Robinia pseudoacacia* seed bait stations. The footage here clearly shows the mice eating the *R. pseudoacacia* seed, while it's not confirmed whether the Cape bunting consumed any.

2.3.6. Population Density Surveys

There was a significant negative non-linear relationship between the DBH and tree density (figure 2.11.) following a logarithmic curve ($R^2 = 0.77$). Undisturbed sites had lower density (< 0.61 trees/m^2) and larger max DBH (up to 25.67 cm) than disturbed sites which had DBH < 10.5 cm and density > 0.48 trees/m^2.

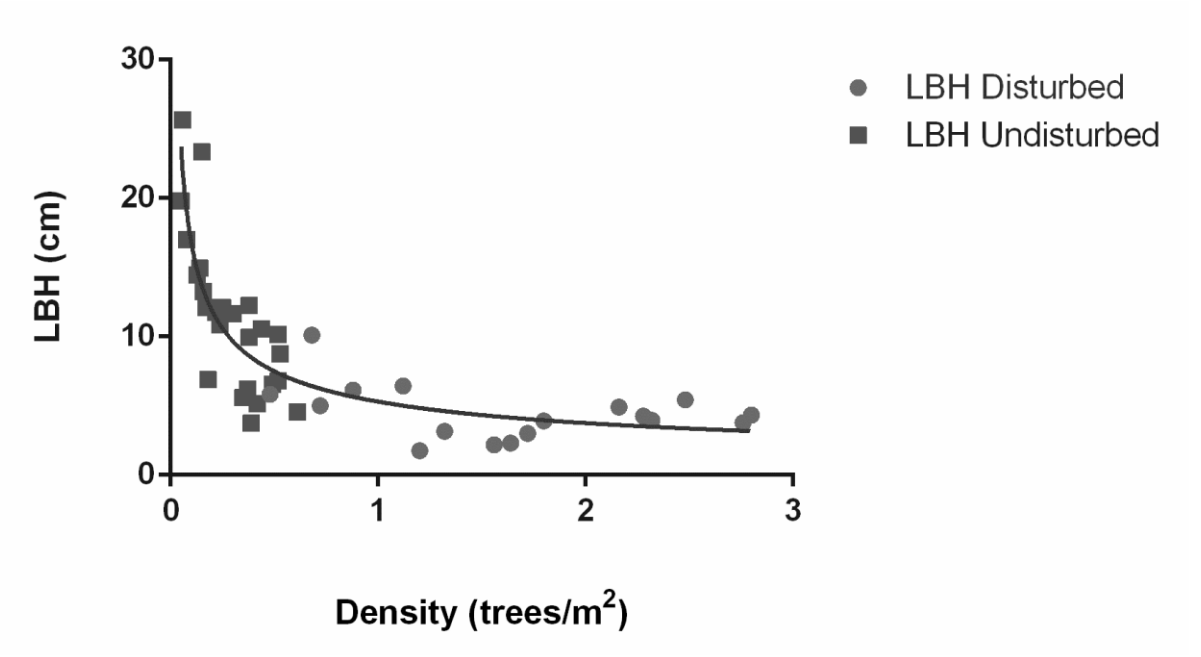

Figure 2.11. Length at breast height (DBH cm) compared to tree density (trees/m^2) illustrating a negative non-linear relationship with a R^2 of 0.77 following a logarithmic curve. Squares indicating trees from disturbed sites and Circles indicating trees from undisturbed sites.

According to a Mann-Whitney test, there is significant difference (P value < 0.0001) between the average DBH of individual trees within undisturbed *R. pseudoacacia* stands compared to the average DBH of individuals from disturbed stands (figure 2.11) with DBH significantly higher at undisturbed sites. Another Mann-Whitney test confirms that there is significant difference (P value < 0.0001) between the population densities of disturbed *R. pseudoacacia* stands versus undisturbed stands (figure 2.12 b).

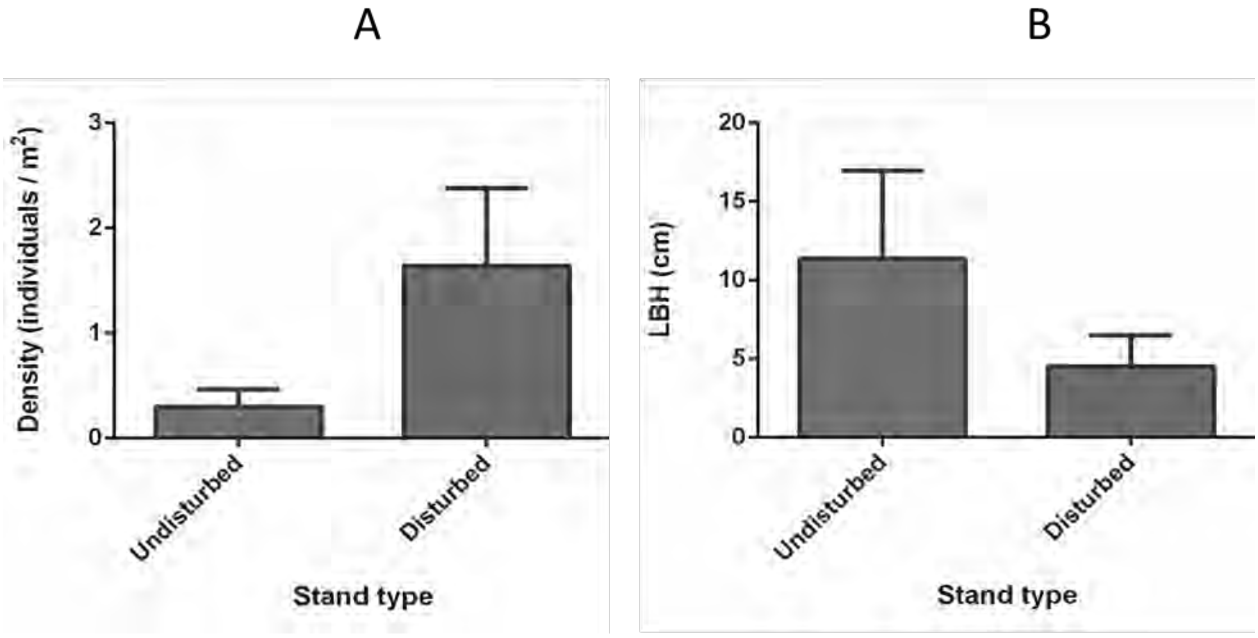

Figure 2.12. (A) The difference in population density between disturbed and undisturbed *Robinia pseudoacacia* stands. (B) The average difference in DBH (Length at Breast Height) between disturbed and undisturbed *Robinia pseudoacacia* stands.

2.4. Discussion

A study conducted by Moran and Southwood (1982) found 105 arthropod species on *R. pseudoacacia* in South Africa. This was not the case during this study. Most arthropods found on *R. pseudoacacia* were also identified as generalists passing through or as predators. This confirmed that no herbivores associated with *R. pseudoacacia* in its native range were found at the sampling sites in South Africa.

Seed surveys were considered to be the highest priority as biological control programmes often prioritise seed-feeding insects first. Seeds were however found to be damaged and left unviable by generalist insects in South Africa, but there were still viable seeds present and therefore the seedbank was examined. The low density of seed found in the soil cores along with the profuse suckering the tree exhibits after mechanical damage, and the speed at which the tree is able to spread vegetatively via a strong connected root system made targeting a seed-feeding insect as a biological control agent impractical. To determine the reason that the seedbank had relatively low numbers of seed compared to other areas such as Switzerland, camera traps with seed bait stations were set up. The cameras caught four-striped mice feeding on seeds on the ground as well as some seed feeding birds scavenging among the bait stations. This explains why there were low numbers of seeds found in the soil cores.

Leaf damage was therefore examined. The final result hoped for is that the biological control agent slows the photosynthetic potential of the tree, reducing the speed at which it can spread vegetatively. In some cases leaf- feeding insects are the known cause of premature leaf drop, thereby reducing the photosynthetic activity and hence the growing season of the tree (Fodor & Hâruţa, 2009a; Wojciechowicz-Zytko & Jankowska, 2004). From 2019 to 2022 leaf damage was surveyed at 21 sites. During 2019, they were surveyed late February, no survey during 2020 and during 2021 and 2022 during March. This was done at the end of summer, just before *R. pseudoacacia* drops its leaves, in order to observe maximum leaf damage during the summer season. From these sites there was very little damage, most of the leaves were in perfect condition. This was found to be constant during the 3 years of surveys with no significant difference. The assumption is that there are few foliovores in the system that specifically target *R. pseudoacacia*. To look at a larger sample set, trees were sampled from south of Mpumalanga Province in South Africa to the northern part of the Eastern Cape Province. This is where more damage was observed at certain sites, but healthy pristine leaves were observed at other sites. To identify the difference the sites were divided into four types depending on the location and neighbouring vegetation. It became evident that *R. pseudoacacia* trees situated within urban environments containing ornamental plants and roadside trees exhibited the most substantial damage This further supports the assumption that generalists insects in the area feed on *R. pseudoacacia* opportunistically rather than seeking it out. This also supports the finding that there are no insects from the native range of *R. pseudoacacia* that may have become naturalised in South Africa.

To identify how much damage generalists are doing to *R. pseudoacacia* in South Africa compared to specialists in the native range (North America), the total damage from all the sites in South Africa and North America was averaged and compared to one another. Although there is some damage to the leaves in South Africa, it was found that there is significantly more in North America. This indicates that the need for a foliovore biological control agent is valid and that although damage has been observed, there is no indication that it induces premature leaf drop as observed in highly infested areas with specialists in North America. Furthermore, type of damage was examined. In the native range six types of damage were observed compared to South Africa where only one type of damage was seen. This again indicates that there are no specialists of *R. pseudoacacia* in the system and that there is an opportunity and space for a foliovore biological control agent in South Africa.

Population densities were also measured in order to form a baseline on which future surveys can reflect. It was found that the more a site is disturbed by means of mechanical damage the denser the stand becomes. *Robinia pseudoacacia* stands that are left untouched and are not controlled by means of chopping for example are less dense even though they have broader tree trunks (DBH). This strengthens the arguments for biological control, and that mechanical control only worsens the problem.

The study showed that *R. pseudoacacia* population density and dynamics in South Africa are of such a nature that biological control is needed and that foliovores are good candidates with which to start the biological control programme. In Comparison with North America, the native range, revealed significantly less leaf damage in South Africa, emphasizing the need for a foliovore biological control agent to mitigate the invasive nature of black locust in the region.

Chapter 3. Desktop review of potential biological control agents for *Robinia pseudoacacia*

3.1. Introduction

One of the dominant hypotheses used to explain invasive species' success is the enemy release hypothesis. The hypothesis suggests that a species that has been moved out of its native range to an area where it is free from coevolved herbivores has a competitive advantage over native species (Keane & Crawley, 2002). The intention of a classical biological control programme is therefore to release natural enemies from the plant's native range into its invaded range to suppress its spread and reduce its population size (Sheppard *et al*., 2006). Plants within their natural range have more than one insect species attacking them, i.e., potential biological control agents. Selecting the best candidate biological control agent from those species is an important step within any biological control programme as the most damaging candidates need to be selected as they will, at first, be the only specialist herbivore present on the invasive weed (Wapshere, 1975). These agents must be specific to the weed but also effective, meaning they have to cause enough damage to suppress the weed (Sutton *et al*., 2021). Predicting efficacy is especially challenging when selecting an agent, especially when selecting one insect among many attacking the plant (Sutton *et al*., 2021). By weighing the potential benefits of introducing biological agents into novel environments against the potential risks and costs, biological control practitioners determine whether or not a potential biological control agent should be released. Choosing the right agent has always been a complex question for biological control practitioners. Some suggest a single agent 'silver bullet' type of approach. For example, a single biological control agent controls the water weed *Azolla filiculoides* Lam. (Salviniaceae) in South Africa (Hill & Coetzee, 2017). Whilst the 'shotgun' approach, such as the biological control of water hyacinth, *Pontederia crassipes* Mart. (Pontederiaceae), has eight agents established in South Africa (King, 2014; Coetzee *et al*., 2021). Nonetheless, a certain level of selection needs to take place before an agent can be released. There are different methods, criteria, and resources available for the prioritization of biological control agents, and there have been attempts to formulate more objective methods for choosing agents using scoring systems or models (for e.g., see Harris, 1973; Van Klinken & Raghu, 2006). Furthermore, there is an increasing trend in weed biological control science to prioritize species before their introduction into quarantine facilities to save expense, space, and time (Briese, 2005; Morin *et al*., 2009).

Some scoring systems are based on ecological theories from past biological control attempts, experimental data, and experience, and some have proved very useful (Crawley, 1989; Sheppard *et al*., 2003; Van Klinken & Raghu, 2006). However, their application risks oversimplification and neglecting valuable agents by being prone to counter-argument and counter-examples. For instance, climate in native ranges versus introduced ranges that may be completely similar is never equivalent and differ in important ecological aspects (Van Klinken *et al*., 2003; Van Klinken & Raghu, 2006).

The criteria used in these methods are based on the level of host specificity, distribution, phenology of attack, and size of the insect (Van Klinken & Raghu, 2006). However, it has been argued that these criteria put more focus on agent traits and not enough on the weed's traits (Van Klinken & Raghu, 2006). Van Klinken and Raghu (2006) suggest investigating the plant in the invaded range may provide more information on agent selection.

Studying the population dynamics and ecology of an invasive plant within its native and invaded range can help identify the part of its life cycle that no longer has an ecological constraint. The part of its life cycle that has no control can then be targeted, and potential biological control agents can be prioritized to specifically attack that part (Sheppard in Spafford & Briese, 2003). However, identifying aspects of the plant's morphology that do not contribute to its invasiveness is also important, and agents suitable for those guilds should not be selected. For example, many invasive species may only have a single-sex (e.g., *Pontederia cordata* L. in South Africa) in the invaded range, making agents that target sexual reproductive organs redundant (Sheppard in Spafford & Briese, 2003).

Wapshere (1985) suggested scoring insects using this method to compare their various attributes or relationships with the weed. These scores are then tallied and used as an estimate of how effective the insect would be as a biological control agent (Harris, 1973; Wapshere, 1985). These criteria consist of feeding damage, fecundity, voltinism, vulnerability, taxonomy, abundance, field host range, climatic matching, genotype matching, and mode of damage (Blossey, 1995; Paterson, 2010). Most of these types of approaches have limited practical use and should be viewed with caution (Van Klinken & Raghu, 2006). However, historically, one of the limitations of these methods is that they are often not possible at the start of a biological control program. There are usually insufficient data about the target plant to determine which aspect of its ecology should be prioritized, or there are insufficient data about the insects associated with the target plant in order to prioritize them.

Interestingly, this is not true when considering *Robinia pseudoacacia* for biocontrol in South Africa, as *R. pseudoacacia* is an important silviculture tree in the United States of America, and the insects associated with the tree have been extensively studied (refer to chapter 1). It is suggested that biological control programmes can be significantly assisted by a comprehensive understanding of the natural enemies associated with the target plant in the region of origin (Syrett *et al*., 2000; Goolsby *et al*., 2004). Having a greater suite of potential agents to choose from allows researchers to make informed decisions about the most appropriate agent for the target weed, ultimately reducing the risk of introducing unsuitable, ineffective agents (Harris, 1973; Goolsby *et al*., 2004; Van Klinken & Raghu, 2006).

One of the final ways of prioritizing agents is by success in other countries' "Piggyback" or "transfer" projects, where biological control agents are imported from the introduced range usually where they have already been shown to be effective (Byrne *et al*., 2021). This allows researchers to benefit from seeing the impact and specificity of an agent once released into an invaded range (either as a biological control agent or an unintentional release (See Byrne *et al*., 2021 for examples). This method has an extensive history in global biocontrol programmes where agents that have been shown to be specific and damaging are released in numerous countries. For example, *Cyrtobagous salviniae* (Coleoptera: Curculionidae) has been released in 20 countries (Winston & Schwarzlander, 2018). In this regard, Europe is a good location to study biological control options for *R. pseudoacacia*. *Robinia pseudoacacia* was first imported into France in 1601, and by 1984 over a million hectares as plantations had been planted in Europe alone (Boer, 2013; Martin, 2019). However, during that time, insects associated with the tree in America also escaped their native predators and managed to reach Europe. The first insect associated with *R. pseudoacacia* that was recorded in Europe was *Euura tibialis* New. (Hymenoptera: Tenthredinidae), which was first recorded in 1825 (Mally *et al*., 2021). *Parectopa robiniella* Clem. (Lepidoptera: Gracillariidae) was then later recorded in 1970, while *Appendiseta robiniae* Gil. (Aphididae) was recorded in 1979. Later, in 1983, *Macrosaccus robiniella* Clem. (Lepidoptera: Gracillariidae) was recorded, and finally, in 2003, *Obolodiplosis robiniae* Hald. (Diptera: Cecidomyiidae) was recorded feeding on *R. pseudoacacia* (Mally *et al*., 2021). Hence, despite not being introduced through official biological control initiatives, it remains possible to examine the effects and selectivity of these insects in Europe. Furthermore, due to the mixed sentiments towards *R. pseudoacacia* on the continent, certain insects have gained notable consideration as significant introduced pests. Consequently, aspects such as their rate of propagation, influence, and connected parasitoid species have been extensively scrutinized (Mally *et al.*, 2021).

In addition to scientific research, the global trend of citizen science platforms allows researchers to access additional data not previously available to biocontrol researchers. There are vast amounts of data that non-professional scientists or volunteers are collecting, which can contribute to scientific knowledge, especially in the field of wildlife and invasion biology (Frigerio *et al*., 2018; Johnson *et al*., 2020). This can also be used to prioritize potential biological control agents. The community platform iNaturalist is especially useful in identifying localities of potential biological control agents (Putman *et al*., 2020). Using this platform, for example, saves time and resources, as biological control practitioners would not have to physically sample large areas. It also provides a temporal perspective which could never previously be achieved through surveys. There are downsides to citizen science as well; for example, there are no dedicated surveys, so species are only located when they are detectable to the general public, consequently, small populations may, unfortunately, be missed. However, it is an important platform whose beneficial attributes are increasingly recognized (Frigerio *et al*., 2018).

As *R. pseudoacacia* has invaded Europe since the 1700s, it is well studied, and due to specialist herbivores of this tree escaping to Europe as well, it gives biological control practitioners excellent insight into the effects these insects have on native vegetation and habitat in a novel environment. In its native range, *R. pseudoacacia* is also well studied, and there are other natural enemies that could be selected as desirable biological control agents (Sheppard *et al*., 2006). The aim of this chapter is to prioritize potential biological control agents for invasive *R. pseudoacacia* by using available resources.

3.2. Materials and Methods

A literature search was conducted on Google Scholar™ (Date accessed: 2019- 2022) including "*Robinia pseudoacacia*" and "Black locust," followed by relevant keywords, including "invasive alien species," "associated insects," "biological control," "biocontrol," "monophagous," "pests," and "herbivores." In addition, grey literature was obtained from various experts who are currently or have previously worked on *R. pseudoacacia*. The reference lists associated with this literature were also considered. These data sources were used to compile the list of insects associated with *R. pseudoacacia*.

To prioritize insects associated with *R. pseudoacacia*, a scoring system was used. The scoring system (Table 3.2) was adapted from Harris (1973) and populated with data derived from the literature. Some criticism has been mentioned about the scoring system, mostly due to a lack of data for the scores (Crawley, 1989; Syrett *et al*., 2000). However, as mentioned previously, *R. pseudoacacia* is well-studied as an economically important plant and as an invader; thus, there are enough data on the tree and its associated herbivores to use the scoring system in an effective manner. The scoring system is also set up in such a way that it is easily modified to include all relevant data, and it also gives results for each insect as an individual (Johnson *et al*., 2020).

There are 12 criteria used in scoring the potential candidates from the system created by Harris (1973). The first criterion looks at host specificity, giving the highest score to oligophagous species with 3 points and the lowest score to restricted monophagous species with 0 points. Although, in this case, monophagous insects were given the highest score, an insect that only feeds on the target species is required. The second criterion considers direct damage inflicted by the insect. The prevention of seed production is the highest-scoring criterion, followed by the destruction of the weed's support system (vascular and mechanical). Only considering leaf damage, insects that can defoliate the weed receive two points. Indirect damage where insects render the weed susceptible to other organisms, for example, disease transmission, with 3 points is the third criterion. The fourth category examines the phenology of the attack. The highest-scoring option with 4 points is a prolonged attack by the insect over the entire growing season. The fifth category examines the number of generations. Obligate univoltine species receive no points, while species that usually have over four generations per year receive 4 points. The sixth parameter pertains to the offspring count generated by the insect in each generation. If the count is under 500, no points are awarded; however, a count exceeding 1000 earns 2 points. As there is an absence of literature data concerning any of the insects, the criterion has been excluded (Van Klinken

& Raghu 2006). Extrinsic mortality factors make up the seventh criterion. If the insect is controlled by non-specific enemies or ecological factors in its native range, it receives no points. If it is immune to non-specific enemies and only controlled by specialized enemies and/or diseases, it receives the highest possible points, 4 (Van Klinken & Raghu 2006).

The eighth criterion is the insect's feeding behaviour. The two options are solitary feeders, 0 points, or colonial feeders, 2 points (Van Klinken & Raghu 2006). The ninth criterion takes into account compatibility of the insect with other biological control agents. If it restricts the possibility of introducing additional agents, it receives 0 points. Otherwise, it receives 2 points for good compatibility. This criterion was also omitted because the insects have never been used as biological control agents. Therefore, there are no data even though they do coexist (Van Klinken & Raghu 2006). The tenth criterion looks at the insect's distribution compared to the weed's range. If it covers the entire range of the weed in its native habitat, it receives the most points, 6 (Van Klinken & Raghu 2006). The eleventh criterion is whether the insect has been successful as a biological control agent elsewhere. If it controls the weeds in its native range, it receives 4 points, and if it controls the weed in other regions of the world, it receives 6 points. It receives 0 points for failure to control the weed in previous biological control attempts or in its native range (Van Klinken & Raghu 2006). For this we treated the insects which were unintentionally introduced into Europe as "biological control agents" even though they are not formally regarded as such

The final criterion takes the size of the insect into account. Less than 5 mg dry weight receives 0 points, while dry weight over 50 mg receives 4 points. This criterion was modified to take these specific insects into account. The largest insect received 4 points, the insect in the middle received 2 points, and the smallest received 1 point (Van Klinken and Raghu 2006). If a certain criterion is unknown about a specific insect, it is left as a blank (-). Another criterion was then added that gave 0 points to the insect if it had a known conflict of interest and 10 points if it had no conflicts of interest (Van Klinken & Raghu 2006). The prioritization system could only be used on insects where sufficient information was available or reasonable inferences could be made (Van Klinken & Raghu 2006).

iNaturalist data were accessed via https://www.inaturalist.org/observations/export (Montgomery, n.d.) (Date accessed: 2019 – 2022). A query was created, and the option to select certain criteria was made available. Research grade "observations verified by experts" was selected. After previewing to ensure selection of the correct data, more options were given, and latitude and longitude were chosen as well. The data were then exported. Some data points only had damage on the leaves, indicating the insect had been there, while other data points had photographs of the insect itself. Data from the insects' native range (North America) and introduced ranges (Europe) were combined and used as one data set to contain the entire northern hemisphere (Refer to chapter 4).

When collecting data from diverse sources like Google Scholar, iNaturalist, and GBIF, potential biases and limitations arise that can impact the overall accuracy and representativeness of the dataset. Spatial bias may occur due to uneven research or citizen science activity, leading to overrepresentation of certain geographical regions. Taxonomic bias is a concern, as some taxa may be extensively studied while others are underrepresented, creating an incomplete biodiversity picture. Variability in data quality and reliability exists, with potential inconsistencies and errors in user-generated content compared to peer-reviewed literature. Sampling bias may occur as a result of voluntary contributions, potentially favouring more visible or accessible species. Temporal bias, influenced by the fluctuating availability of data, further complicates accurate trend assessments. These limitations were acknowledged and mitigated through complementary data collection methods, validation procedures, and standardized practices to enhance the robustness of the collected information (Frigerio *et al.*, 2018).

3.3. Results

3.3.1. Species Lists

Using a variety of references and citizen science platforms, a list was developed of insects associated with *R. pseudoacacia* (Appendix 1). These include 12 borers from the families Buprestidae, Scolytidae, Olethreutidae, Cossidae, Nitidulidae, and Cerambycidae: three galling insects from the families

Olethreutidae and Cecidomyiidae. Thirty-five leaf-feeding insects from the families Phasmatidae, Megalopygidae, Pyralidae, Tortricidae, Oecophoridae, Gracilariidae, Psychidae, Geometridae, Arctiidae, Noctuidae, Notodontidae, Saturniidae, Hesperiidae, Scarabaeidae and

Chrysomelidae. Nineteen sucking insects originated from the families Miridae, Pentatomidae, Membracidae, Cicadellidae, Flatidae, Acanaloniidae, Diaspididae and Coccidae. Two seed predators from the Curculionidae family and four general predators from the Reduviidae and Formicidae families (Figure 3.1) (Hargrove, 1986; Kodoi *et al*., 2003; Smith, 2008; Rasplus *et al*., 2010).

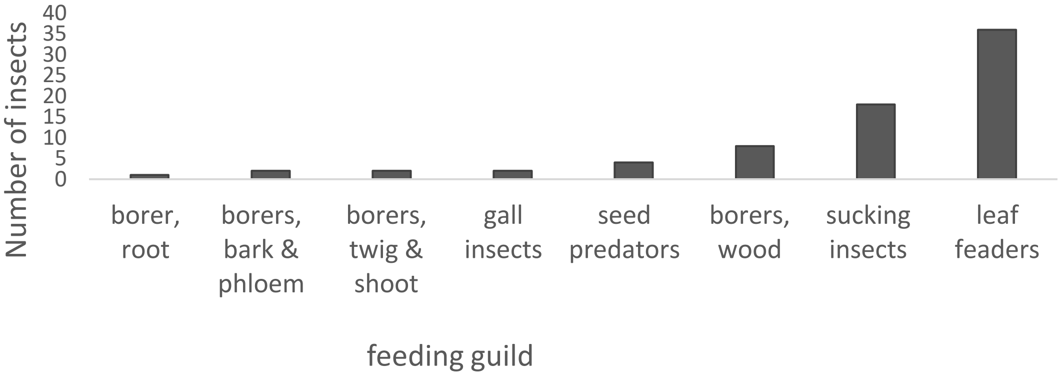

Figure 3.1. Phytophagous insects associated with *Robinia pseudoacacia* including new associations.

Referring to table 3.1, insects were assigned priority. Low priority was given to insects that either do not have enough information to verify if they are monophagous or are known to be polyphagous. Medium priority was given to species known to be monophagous but not abundant in the USA. High priority was given to species known to be monophagous that were also abundant in the USA. A high priority level was also given to insects present in Europe. In biological control, feeding guild is important and should be considered when prioritizing insects as potential agents. These include seed predators, sucking insects, leaf feeders, galling insects, root borers, twig and shoot borers, wood borers, cankers, fungal pathogens and bark and phloem borers.

Insects with high priority were then ranked according to feeding damage and guild specific to leaf feeders, sucking insects and gall insects represented in table 3.1. Insects that are monophagous and are found in high densities within the native range were indicated with asterisks.

Table 3.1. Insects associated with *Robinia pseudoacacia* that are considered a high priority due to being specific to *Robinia pseudoacacia* and being specialised foliovores (* High priority leaf- feeding insects that are monophagous and abundant in the native range)

Family	**Species**	**Common Name**	**Guild**	**References**
Cecidomyiidae	*Dasineura pseudacacia*	locust midge	gall insects	Hargrove, 1986
Cecidomyiidae	*Oblodiplosis robiniae**	locust gall midge	gall insects	Hargrove, 1986; Kodoi *et al*., 2003
Chrysomelidae	*Odontota dorsalis* *	locust leafminer	leaf feeders	Hargrove, 1986; Day, 2016
Membracidae	*Thelia bimaculata*		sucking insects	Hargrove, 1986
	Vanduzeea arquata		sucking insects	Hargrove, 1986
Miridae	*Lopidea heidemanni Knight*		sucking insects	Hargrove, 1986
	L. robiniae		sucking insects	Hargrove, 1986
Tenthredinidae	*Nematus abbotii**		leaf feeders	Hargrove, 1986; Smith, 2008
Tenthredinidae	*Euura tibialis**	locust sawfly	leaf feeders	Hargrove, 1986; Rasplus *et al*., 2010
Giadlariidae	*Macrosaccus robiniella**		leaf feeders	Hargrove, 1986; Bakay & Kollár, 2014
Giadlariidae	*Parectopa robiniella**	locust digitate leaf miner	leaf feeders	Hargrove, 1986; Baugnée, 2014

Cerambycidae	*Megacyllene robiniae*	locuts borer	Stem miner	Galford, 1997; Sheppard *et al*., 2006

There are some pathogens associated with *R. pseudoacacia* as well. *Phloeospora robiniae* (Lib.) Höhn. (Mycosphaerellaceae) affects the leaves causing necrosis and deformation starting in early spring. *Phyllosticta advena* (Botryosphaeriaceae) causes black leaf spots during late summer (Wilkaniec *et al*., 2021). Other pathogenic fungi also associated with *R. pseudoacacia* include *Erysiphe polygoni* (Vaňha) Weltz. (Erysiphaceae) and *Phyllosticta robiniae* Pers. (Botryosphaeriaceae) (Wilkaniec *et al*., 2021). Pathogenic fungi were, however, not within the scope of this study.

The locust borer *Megacyllene robiniae* Forster (Coleoptera: Cerambycidae), was also considered as it is a major pest of *R. pseudoacacia* trees (Galford, 1997; Sheppard *et al*., 2006). This borer is large, monophagous and widespread throughout its host's native range, however, it is univoltine and the damage it inflicts on the tree often results in "clump growth" (Galford, 1997). This is due to root suckering, which is the tree's response to mechanical damage, leading to increased spread of the weed (refer to chapter 1).

3.3.2. Prioritisation

To narrow down the list in Table 3.1 and to identify the agent most likely to be successful, a scoring system was used. The scoring system (see Table 3.2) was adapted from Harris (1973) based on existing literature on insects associated with *R. pseudoacacia*.

From pre-release field surveys conducted to study the population dynamics of *R. pseudoacacia* in South Africa, it was determined that the tree spreads mostly by root suckering and less by seed dispersal (Chapter 2). Leaf-feeding insects were therefore identified as a priority, excluding seed feeders and borers before using the scoring system. Borers were excluded during this study due to the unknown reaction the tree would have to mechanical-type damage. Further research would have to be done first to eliminate the possibility that a boring insect would prompt suckering, a reaction *R. pseudoacacia* has to mechanical damage.

Table 3.2. Effectiveness scores of the selected leaf-feeding insects to determine the best suitable biological control agent for *Robinia pseudoacacia* (Harris, 1973). Six potential biological control agents were ranked according to 11 criteria, namely host specificity, direct and indirect damage inflicted, phenology of attack, number of generations, extrinsic mortality factors, feeding behaviour, distribution, effectiveness, size, and conflict of interest.

	Obolodiplosis robiniae	***Odontota dorsalis***	***Euura tibialis***	***Nematus abbotii***	***Macrosaccus robiniella***	***Parectopa robiniella***
1. Host specificity	3	1	1	1	3	3
2. Direct damage inflicted	0	2	0	0	0	0
3. Indirect damage inflicted	0	3	0	0	0	0
4. Phenology of attack	1	2	1	1	2	1
5. No. of generations	4	2	2	0	4	2
6. Extrinsic mortality factors	4	4	-	-	0	0
7. Feeding behaviour	2	2	-	-	2	0
8. Distribution	6	6	6	4	6	6
9. Effectiveness	4	4	4	-	4	4
10. Size	1	4	2	2	1	1
11. Conflict of interest	10	10	0	0	10	10
Total	**35**	**40**	**16**	**8**	**32**	**27**

3.3.3. Final Species

The final species proposed as the most likely suitable biological control agents are listed below, from most to least suitable. Each potential biological control agent also has a figure compiled using data from iNaturalist, where users have recorded the time of year the insect was identified. These figures illustrate the best sampling times for the potential biological control agents of this study, thus demonstrating one of the advantages of using citizen science. Furthermore, they establish which insects have the longest period of activity during the year.

3.3.3.1 *Odontota dorsalis (*40 points)

The first potential biological control agent is *Odontota dorsalis*, commonly known as the locust leafminer. It occurs throughout the middle and eastern United States, east of the Mississippi River (Fritz, 1983; Wheeler Jr, 1980), and *Robinia pseudoacacia* is its preferred host (Wheeler Jr, 1980; Kirkendall, 1984; Hargrove, 1986; Day, 2016). Although adults have occasionally been found on soybeans, apples, elms, oaks, birches, beeches, cherries, hawthorns, honey locusts, and Japanese pagoda trees (USDA, n.d.; Wheeler Jr, 1980), adult damage is minor compared to the extensive damage done by the larvae. This has not been recorded on the above non-target plants. (Fritz, 1983; Day, 2016). *Odontota dorsalis* larvae, the primary herbivore for *R. pseudoacacia*, are able to completely defoliate the tree when they reach high densities between July and August in the Northern Hemisphere (Fritz, 1983; Athey & Connor, 1989). The defoliation by the larvae substantially reduces the growth and flowering of *R. pseudoacacia* (Fritz, 1983), and occasional tree mortality occurs when outbreaks of *O. dorsalis* are combined with other stress factors (USDA, n.d.).

Adults chew holes through the upper surface of the leaflets (Figure 3.2), while larvae mine the mesophyll, which eventually coalesces, causing leaves to turn brown and fall (Fritz, 1983; Day, 2016). Numerous leaves may be attacked by a single leafminer (Day, 2016). Adults overwinter within the bark fissures or in the leaf litter of *R. pseudoacacia* and emerge in spring as the leaves unfold (USDA, n.d.). Three to five overlapping eggs are deposited from mid-May to mid-June on the underside of the leaflet surface and are cemented with excrement (USDA, n.d.; Wheeler Jr, 1980). Early instar larvae may be found in a single mine, but late instars disperse and excavate their own from which they pupate in July (USDA, n.d.; Wheeler Jr, 1980). Two generations develop per year (Wheeler Jr, 1980).

Adult beetles are flat, elongated, and measure about 6 mm in length. The head is black, while the wings, thorax, and elytra are orange with a recognizable black dorsal stripe running down the middle (USDA, n.d.; Day, 2016). The eggs are flat, white, and oval, and the larvae are yellow (USDA, n.d.; Day, 2016).

Figure 3.2. *Odontota dorsalis* adult beetles and the damage they do to the surface of the leaves (iNaturalist, 2022).

Odontota dorsalis scored the highest with 40 points according to the Harris (1973) assessment. It is also an attractive option as a biological control agent. It has ample geographic records according to iNaturalist, however only in the native range. A potential candidate that has escaped its native range has the advantage of more research being done as it is often considered a pest. A potential biological control agent that is only found in its native range has no data on how it adapts to foreign environments and climates. It is however abundant in its native range and according to literature inflicts the most damage to *R. pseudoacacia*. In its native range it is abundant from March to November with a peak in June (figure 3.3).

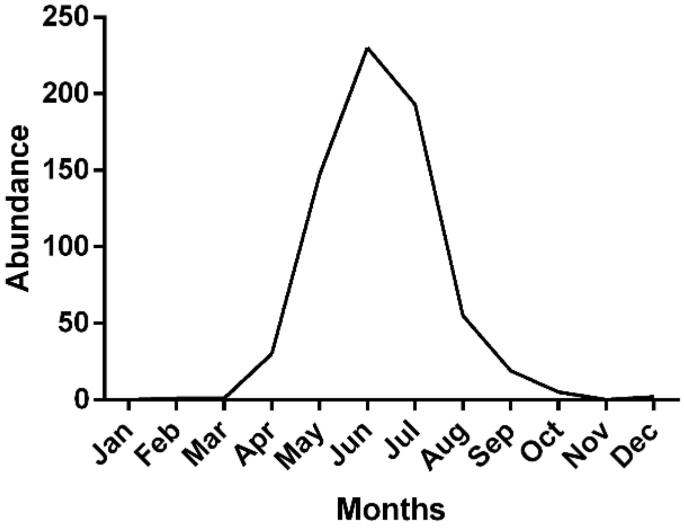

Figure 3.3. Adult *Odontota dorsalis* abundance in the field in USA (iNaturalist, 2022).

3.3.3.2 *Obolodiplosis robiniae* (35 points)

Obolodiplosis robiniae (Haldeman) is commonly known as the locust gall midge. This species is a promising prospective biological control agent partly because most galling insects are unable to expand their host range to other species (Kodoi *et al*., 2003). It has also escaped its native range with its host and is found in higher densities in its invaded range than in its native range according to citizen science surveys. South Korea was the first country where *O. robiniae* was identified outside its native range in 2002 (Kodoi *et al*., 2003; Weryielinger & Skuhrava, 2007; Duso *et al*., 2011). One of the reasons it received a lower ranking than *Odontota dorsalis* is that it is known to be parasitized by Platygastridae (Hymenoptera: Proctotrupoidea) in Europe and in Asia, which should be taken into consideration when considering the insect (Duso *et al*., 2011). However, despite the parasitism, its numbers do not seem to be affected within the invaded range.

Its biology within its native range is poorly studied, but it has been extensively studied outside its native range (Duso *et al*., 2011). Studies from Asia and Japan indicate that *O. robiniae* is multivoltine (Molnar *et al*., 2009; Tóth *et al*., 2009; Duso *et al*., 2011) and develops two to four generations per year (Kodoi *et al*., 2003; Tóth *et al*., 2009; Duso *et al*., 2011), with overlapping generations (Tóth *et al*., 2009;

Skuhravá *et al*., 2015). This is due to the fact that altitude, temperature, and prolonged periods of egg-laying influence the development of *O. robiniae* (Skuhravá *et al*., 2015). Females lay their eggs on the young leaflets of mature *R. pseudoacacia* trees, particularly at the terminal parts of the shoots, and then the larvae pupate in rolled leaf margins (Figure 3.4) (Tóth *et al*., 2009; Duso *et al*., 2011; Shang *et al*., 2015). The larvae cause the margins of the leaflets to thicken and bend downwards, forming the distinctive leaf margin roll (Weryielinger & Skuhrava, 2007), as they start to attack young shoots growing from the roots (Shang *et al*., 2015). Each gall can contain five to six larvae, and it changes colour from green in the beginning to red as it dries up (Tóth *et al*., 2009). Multiple generations of galls can be observed on a single leaflet when larvae are found at high densities, and the leaflets are rolled along the midrib (Tóth *et al*., 2009; Duso *et al*., 2011). The more larvae there are living in the galls, the more damage there will be to the leaf, and at high infestations this can result in defoliation of the *R. pseudoacacia* (Sheppard *et al*., 2006; Tóth *et al*., 2009; Duso *et al*., 2011), although the tree is able to regrow these leaves within the season (Sheppard *et al*., 2006).

Figure 3.4. *Obolodiplosis robiniae* adult midge and the manner in which they roll *Robinia pseudoacacia* leaves where the larvae feed and pupate (iNaturalist, 2022).

Eggs are light orange in colour and become darker and red before they hatch (Park *et al*., 2009). They are oval-shaped and measure 0.4 mm in length. The larvae measure 2.6 mm and are a milky white colour (Park *et al*., 2009). The pupa is characteristically brown and measures 3.2 mm (Park *et al*., 2009). The wings of adults are black while their abdomens are reddish. Female midges are larger than males and measure about 3.3 mm (Park *et al*., 2009). In the Northern Hemisphere, the emergence of *O. robiniae* starts from late April to mid-May and ends around mid-November with overlapping generations in between (Figure 3.5) (Park *et al*., 2009; Tóth *et al*., 2009). In a study by Park *et al*. (2009), it was observed that the first emerged adult pupated in the soil while the second and third emerged adults pupated in the galled leaf directly. This is because the larvae of the last generation leave the galls to overwinter and pupate in the soil (Tóth *et al*., 2009; Duso *et al*., 2011). The midge can be found on trees of different ages and sizes within natural stands, along roads, agroecosystems, and gardens and is found throughout the distribution of its host regardless of altitude (Duso *et al*., 2011).

This potential agent scored 35 points according to the Harris (1973) assessment. It was the second-highest score out of the six candidates. It is a very attractive candidate as it has many verified geographic records on iNaturalist in the invaded range (mostly Europe), where it is considered a pest (Molnár *et al*., 2009). According to sightings from iNaturalist, it is also active for most of the year. This information may also indicate that it is abundant and easy to find as there are so many geographic and multi-seasonal records.

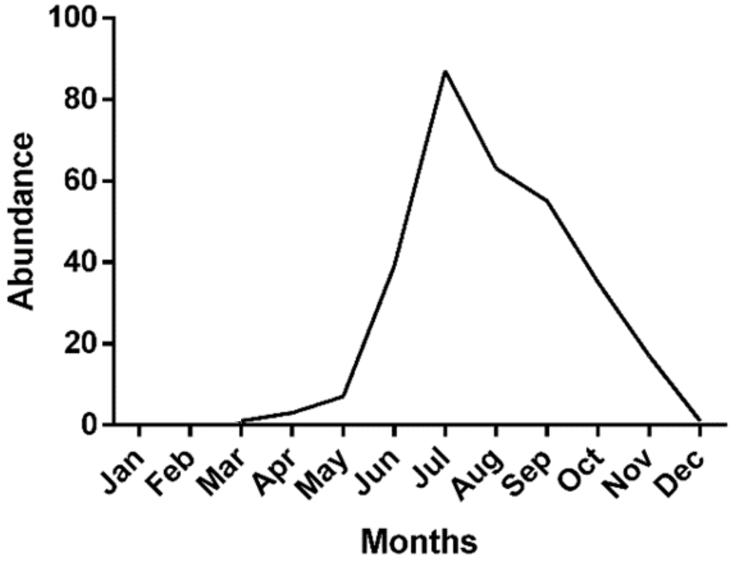

Figure 3.5. Adult *Obolodiplosis robiniae* abundance in the field in USA (iNaturalist, 2022).

3.3.3.3. *Macrosaccus robiniella* (32 points)

Macrosaccus robiniella has two to three, sometimes up to four, overlapping generations per year, depending on the climate. These generations take five to eleven weeks to complete, ending when the last generation moths go into diapause under and within bark crevices (Wojciechowicz-Zytko & Jankowska, 2004; Stojanović & Marković, 2005; Ureche, 2006; Ivinskis & Rimšaite, 2008; Csóka *et al*., 2009; Bakay & Kollár, 2014).

Moths oviposit at the end of May or beginning of June when *R. pseudoacacia* has new leaves. The first generation is then found from mid-June to beginning of August in the Northern Hemisphere (Whitebread, 1990; Wojciechowicz-Zytko & Jankowska, 2004; Stojanović & Marković, 2005). Second generation moths can be found from mid-July or beginning of August to mid-September, while third generation moths can be found from mid-August to mid-May before hibernation (Wojciechowicz-Zytko & Jankowska, 2004; Stojanović & Marković, 2005).

When the leaves of *R. pseudoacacia* first appear in May, females oviposit on the undersides of the leaves, away from the vein, where caterpillars form their initial tunnels after hatching. Several eggs are oviposited per leaf on either side of the midrib, and it is possible to find up to twelve caterpillars mining a single leaflet (Whitebread, 1990; Wojciechowicz-Zytko & Jankowska, 2004; Stojanović & Marković, 2005). The larvae hatch after six to ten days and immediately start to mine, creating elongated oval-shaped blotches under the epidermis of the leaf's surface (see figure 3.6) (Wojciechowicz-Zytko & Jankowska, 2004; Stojanović & Marković, 2005). Larvae are 4.5 mm and light green with a brown head, and they are gregarious feeders (Whitebread, 1990; Ureche, 2006). Their development may take anywhere from 20 to 50 days, depending on the climate (Stojanović & Marković, 2005). The mines then reduce the tree's ability to photosynthesize and may cause it to shed its leaves prematurely when the insect is present at high densities (Stojanović & Marković, 2005), which would be the ideal outcome from this potential biological agent. Pupation then takes place in a flattened white silky cocoon (Whitebread, 1990).

Adults have a wingspan of 5 to 6 mm and are brown and beige patterned (Wojciechowicz-Zytko & Jankowska, 2004; Ureche, 2006). However, several parasitoids are associated with *M. robiniella* belonging to the Eulophidae and Braconidae families (Stojanović & Marković, 2005).

Figure 3.6. *Macrosaccus robiniella* adult moth and how they damage *Robinia pseudoacacia* leaves where the larvae feed and pupate (iNaturalist, 2022).

Macrosaccus robiniella scored 32 points according to Harris (1973). It is therefore also a suitable candidate to investigate further. It is also quite similar to *O. robiniae* in terms of geographic records on iNaturalist as it has escaped from its native range and is considered a pest in its invaded range. It is also quite abundant for most of the year in regions that are accessible due to the number of recorded locations by civilians from March to December (figure 3.7). These results support further research.

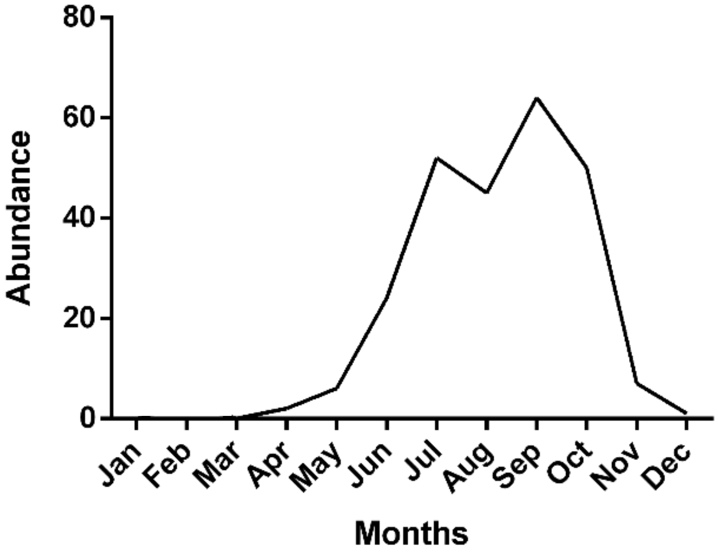

Figure 3.7. Adult *Macrosaccus robiniella* abundance in the field in USA (iNaturalist, 2022).

3.3.3.4 *Parectopa robiniella* (27 points)

Parectopa robiniella is also native to the United States and is commonly known as the locust digitate leaf miner, referring to the finger-like shape of the mines (Baugnée, 2014; Martin, 2019). Similarly to *M. robiniella*, it was accidentally introduced in Europe where it was first recorded in Italy in 1970 (Whitebread, 1990; Csóka *et al.*, 2009; Baugnée, 2014). It gradually spread throughout Europe, reaching at least 19 European countries (Csóka *et al.*, 2009; Fodor & Hâruţa, 2009; Baugnée, 2014). According to Hargrove (1986), *M. robiniella* is one of the main herbivores of *R. pseudoacacia* and is considered an oligophagous leaf miner. It has been observed on other Fabaceae species within its native range, but in its escaped range, it has only been found on *R. pseudoacacia* (Baugnée, 2014; Fodor & Hâruţa, 2009).

Parectopa robiniella is bivoltine but may have up to three generations per year, depending on the climate. Adult moths are present from May to September in the Northern Hemisphere, while being active from mid-March to December (Figure 3.9) (Ureche, 2006; Csóka *et al*., 2009; Baugnée, 2014). Larvae are solitary feeders with only one larva per mine and develop two to three weeks after *M. robiniella* (Ureche, 2006; Ivinskis & Rimšaite, 2008; Csóka *et al*., 2009). Females oviposit on the topside of the leaf surface, where mines become apparent as green-coloured larvae begin to develop (Baugnée, 2014). Pupae are then found in white cocoons outside the mines, either on the leaves or in the leaf litter, depending on the generation (Figure 3.8) (Baugnée, 2014).


Parectopa robiniella

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Figure 3.8. *Parectopa robiniella* adult moth and the manner in which they damage *Robinia pseudoacacia* leaves where the larvae feed and pupate (iNaturalist, 2022).

Parectopa robiniella causes premature leaf drop, which negatively affects photosynthesis and has caused notable damage to cultivated *R. pseudoacacia* (Ivinskis & Rimšaite, 2008; Fodor & Hâruţa, 2009; Baugnée, 2014). However, several parasitoids are associated with *M. robiniella*. In Europe at least 15 species, usually from the family Eulophidae, are known to parasitize *M. robiniella* (Baugnée, 2014).

Parectopa robiniella also scored below 30 points according to the Harris (1973) assessment, with 27 points. It is considered lacking in crucial criteria for a successful biological control candidate, and with other options available, it is deemed unnecessary to further research this insect at this stage.

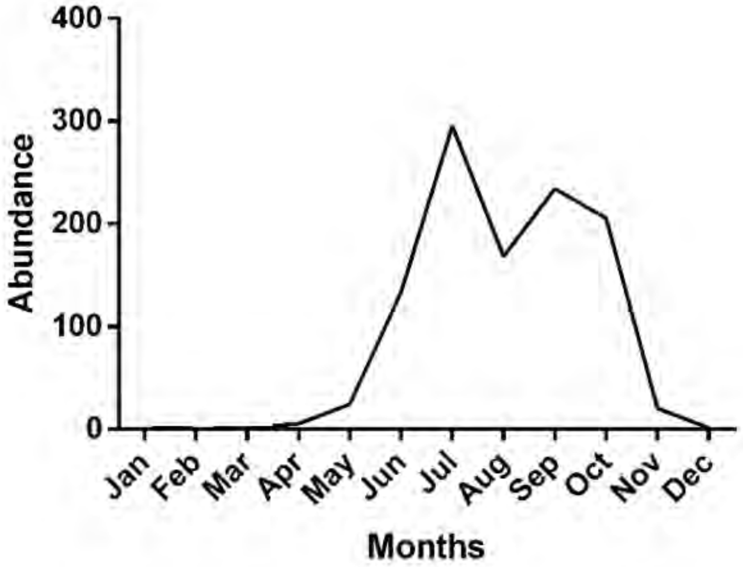


Figure 3.9. Adult *Parectopa robiniella* abundance in the field in USA (iNaturalist, 2022).

3.3.3.4 *Euura tibialis* (16 points)

Euura tibialis (Newman), commonly known as the locust sawfly, is another option and is also an important herbivore of *R. pseudoacacia* (Hargrove, 1986; Bakay & Kollár, 2014). It is native to the United States but has been accidentally introduced into Europe with imported wood and is now considered invasive (Rasplus *et al*., 2010). In its native range, it has also been recorded on *Robinia hispida* and *Gleditsia triacanthos*, trees that are not present and are considered invasive in South Africa, respectively. *Euura tibialis* has more than one generation per year, and adults are active from mid-summer until early autumn (Smith, 2008). The adults are about 6 mm in length and are green, sometimes yellow to brown, with black spots (Smith, 2008). Larvae are green with no unique markings (figure

3.10) (Smith, 2008). Sawfly adults in general are short-lived, taking only a few days to produce eggs (Mahr, 2017). Eggs are deposited within a cut in the leaf that the females make with their ovipositors (Mahr, 2017; ISU, 2020). Larvae develop through six instars before reaching maturity, which may take two to four months (Mahr, 2017). They are active from March to December, with two peaks per year, in June and September (figure 3.11). High densities of sawflies overall can cause substantial damage to their hosts but are not fatal to healthy, well-established trees or shrubs (Mahr, 2017). However, young trees can be completely defoliated, which can stunt or even kill them (Mahr, 2017).

Figure 3.10. *Euura tibialis* adult sawfly and how the larvae damage *Robinia pseudoacacia* leaves where they feed (iNaturalist, 2022).

Euura tibialis scored below 20 points according to the Harris (1973) assessment with 16 points. It's therefore considered a poor candidate for biological control as it lacks many characteristics that ensure a successful biological control agent. Importing this insect and using time and resources in quarantine would be considered wasteful when there are more promising options.

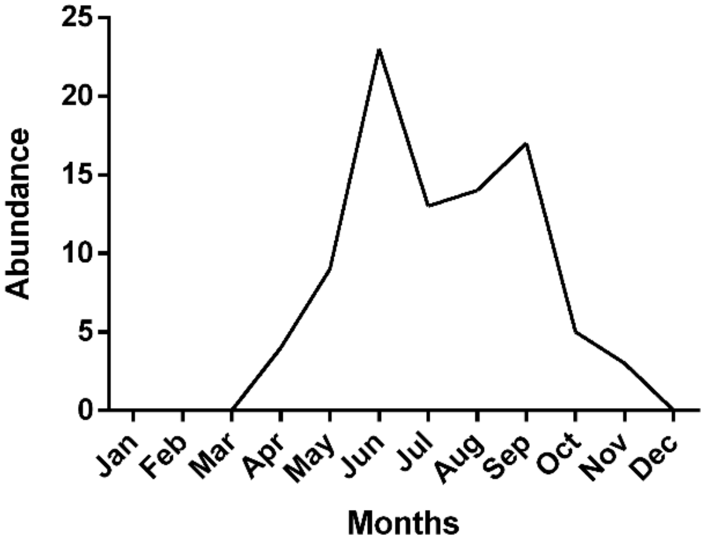


Figure 3.11. Adult *Euura tibialis* abundance in the field in the USA (iNaturalist, 2022).

3.3.3.5 *Nematus abbotii* (8 points)

Nematus abbotii (Kirby) is the last option mentioned in this study. As with *E. tibialis*, the literature on *N. abbotii* biology is sparse (Smith, 2008). It is also a specialist herbivore on *R. pseudoacacia* (Hargrove, 1986) and is abundant where its host is found, particularly in West Virginia, Virginia, and Maryland (Smith, 2008). Adults can be found in April and May during spring, and because they are not abundant during other months, it is deduced that they only have one generation per year (Smith, 2008). *Nematus abbotii* adults are larger than *E. tibialis* and are predominantly black, while their larvae are green with black spots (figure 3.12) (Smith, 2008).

Figure 3.12. *Nematus abbotii* sawfly larvae at different instars and the manner in which the larvae damage *Robinia pseudoacacia* leaves where they feed (iNaturalist, 2022).

Nematus abbotti scored the lowest, with only 8 points, according to Harris' (1973) assessment. According to surveys, they are active from March to July, which is also the shortest active time of the potential biological control species (figure 3.13). As a result, this was given the lowest ranking and should only be considered a priority if alternative agents are dismissed, in order to avoid a wastage of time and resources.

Both sawfly species lost points in the Harris (1973) assessment because they have a potential conflict of interest. The first insect to be rejected as a biological control agent for the Brazilian Peppertree was a sawfly, *Heteroperreyia hubrichi* Malaise (Hight *et al*., 2003; Wheeler *et al*., 2016). Not only did females of this species accept the native plant (*Rhus sandwicensis*) as an acceptable oviposition host (Hight *et al*., 2003), but also the fact that this sawfly species, along with other sawfly species, is known to produce cytotoxic peptides (Wheeler *et al*., 2016). These toxins were found at a concentration that would be of concern for livestock and wildlife, and from numerous studies conducted, it was found that if these insects were to be released as biological control agents, they would pose a threat to that environment (Oelrichs *et al*., 1999,2001; Wheeler *et al*., 2016; Boevé *et al*., 2018; Boevé & Rozenberg, 2019).

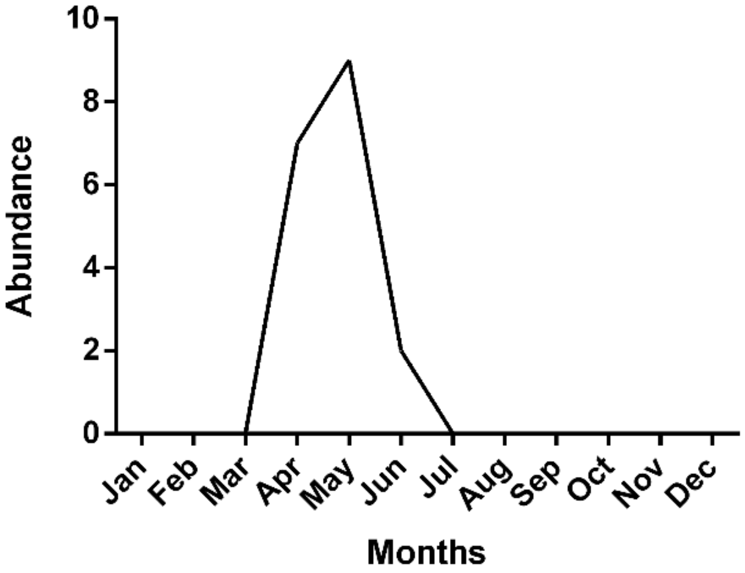


Figure 3.13. Adult *Nematus abbotii* abundance in the field in the USA (iNaturalist, 2022).

3.4. Discussion

3.4.1. Potential for biological control success

With evidence of biological control being effective against weeds in the Fabaceae family worldwide and in South Africa, the prospect of implementing such control on *R. pseudoacacia* appears promising. In the past, it has also been classified as a potential biological control target, but no such effort has been made (Sheppard *et al.*, 2006; Martin, 2019;).

According to the Biological Control of Weeds Catalogue of Agents and their Target Weeds (Winston *et al.*, 2014), 27 species within the Fabaceae have been targeted for biological control. Within those 27 species, 80 unique agents have been considered, and many of those have been successful (see Appendix 2). South Africa has benefited from biocontrol programmes against invasive Fabaceae species, such as invasive Australian acacias and prosopis species. One of South Africa's most well-documented successes is *Sesbania punicea*, a closely related species to *R. pseudoacacia*, which is successfully controlled by three insects (Evans & Rotar, 1987; Hoffmann & Moran, 1991; Wojciechowski *et al.*, 2004). These insects are listed in Appendix 2 with varying levels of success.

3.4.2. Prioritisation of potential biological control agents

Sheppard *et al*. (2006) consider *R. pseudoacacia* to be one of the top 20 weeds suited for classical biological control in Europe, which is also true for South Africa. This is primarily due to its impact, and because four of the species within the Robinia genus found in Europe are known to be invasive outside their native range (North America). It has also recently been ranked as the second-best candidate for biological control in South Africa due to the impact and importance of the target weed and the likelihood of achieving biological control success (Canavan *et al*., 2021).

To release biological control agents on invasive weeds, host specificity tests have traditionally been used to ensure the safety of native vegetation (Fowler *et al*., 2000). Host specificity testing is generally the most time-consuming and expensive part of a biological control program (Fowler *et al*., 2000; Heard & Van Klinken, 2004; Dhileepan *et al*., 2006). There are also factors that influence the success of biological control agents once they are released, and resources are wasted when they are not as successful as predicted (Harris, 1991). As *R. pseudoacacia* and its associated herbivores are extensively studied in both its native and invaded ranges, suitable biological control agents are available that could be prioritized.

Citizen science is developing rapidly and can be increasingly used in science. With the use of citizen science, ideal sampling times and locations from up-to-date distribution records can be determined with high accuracy without wasting time or funds. Seasonal abundance figures demonstrate how valuable citizen science can be with reference to sampling regions and the seasonality of the top three prioritized insects. It shows when the insect has been observed, allowing sampling trips to be scheduled at the right time. Not only does iNaturalist help identify where potential biological control agents are, but it also helps determine the best time of year to schedule collection surveys.

In this chapter, various criteria of prioritization were implemented to identify the most suitable and potentially successful biological control agent. The approach in this study incorporated the process of elimination, similar to the table used in Harris (1973), which indicated viable biological control agents to be studied further. This approach is seldom used in practice today, although it was found to be useful in this study. Citizen science was also used as a novel approach to identifying abundance, potential sampling locations, and suitable sampling time. Citizen science is likely to become a major advantage to biological control practitioners in the future with the amount of data made available by the public. These insects have been proven to be active during the growing season of *R. pseudoacacia*, but not the entire season. Selecting more than one agent will therefore be the most beneficial, so that their active times overlap and target the tree for the entirety of its growing season, as well as targeting the leaves in different manners (rolling, cutting, and surface area damage). Finally, an intensive literature study was used to identify which insects would be the most successful based on their biology and life cycles. During this study, potential flaws were identified, such as the toxicity potential of the sawflies, making them unsuitable as biological control agents.

In conclusion, the three candidates to investigate further are *O. robiniae*, *O. dorsalis,* and *M. robiniella*. Further techniques to be used in the prioritization of these insects include ecological niche and climate modelling, genetic matching, and the use of phylogenetics.

Chapter 4. Predicting suitability for the establishment of potential biological control agents for *Robinia pseudoacacia* in South Africa

4.1. Introduction

This chapter focused on the management of *R. pseudoacacia* in South Africa. Climate models, including correlative (MaxEnt) and mechanistic (CLIMEX) approaches, predicted species distribution based on environmental factors. MaxEnt, preferred for its consistent performance, was highlighted for its use in presence-only data scenarios, requiring tuning of parameters. The chapter further emphasizes the importance of pseudo-absence data in MaxEnt modeling addressing sampling biases, characterizing unsuitable habitats, and preventing overfit models. The aim was to identify potential biological control agents for *R. pseudoacacia* in South Africa based on their ability to thrive in specific climatic conditions, considering both native and invaded ranges.

Climate models, such as CLIMEX, MaxEnt, BIOCLIM, and others, are important tools in ecology and are used for various purposes in ecological research and management (Julien & White, 1997; Tang *et al*., 2021). These models are employed in ecological niche modelling (ENMs) or species distribution modelling (SDMs) to predict the potential distribution of species based on their environmental requirements (Tang *et al*., 2021; Elith *et al*., 2010). Correlative models, including MaxEnt and Boosted Regression Trees (BRT), focus on establishing the relationship between a species and its environment to predict its geographic distribution (Robertson *et al*., 2003; Peterson *et al*., 2015). These models use known species distribution data and environmental variables, such as soil characteristics, temperature, and precipitation, to determine the suitable habitat for a species (Kriticos *et al*., 2021). By correlating the species' distribution with environmental parameters, correlative models can estimate the potential range of a species. On the other hand, mechanistic models, such as CLIMEX, integrate not only environmental requirements but also consider factors like dispersal ability and biotic interactions to simulate the potential distribution of a species (Peterson *et al*., 2015). Mechanistic models aim to capture the fundamental niche of a species by incorporating its physiological limits, tolerances, and interactions with other organisms. These models provide more detailed insights into the potential areas where a species can persist.

In the context of biological control, climate matching models are used to identify suitable regions for introducing potential biological control agents (Julien & White, 1997). By matching the climate conditions of the native range of the target invasive species with potential regions for release, researchers can identify areas where the biological control agents are more likely to establish and control the target species (Sutton, 2019).

Selection of the appropriate model depends on the research objectives, available data, and the ecological processes being studied. Both correlative and mechanistic models have their advantages and limitations, and researchers choose the model that best suits their specific research questions and data availability. Climate matching models are popular in weed management. These models have been used to determine the potential areas weeds can invade (for example Martin *et al*., 2020) as well as where to collect potential biological control agents that are in areas climatically similar to the weeds' invaded range, see for example Sutton (2019). These methods will not be able to predict the impact the agents will have on the weed but will be an indication of climate suitability between the native and introduced range which is expected to lead to increased establishment / abundance. (Julien & White, 1997; Phillips & Dudı´k, 2015; Sutton, 2019).

It is important to consider climate and climate matching when initiating biological control programmes as climate influences the distribution and abundance of plants and animals (Rafter *et al*., 2008; Robertson *et al*., 2008). Studies have shown that if the climate of the introduced range is similar to the species' native range, the introduced species is more likely to thrive in the new region (Julien & White, 1997). Matching the climate of the introduced range with that of the native range increases the likelihood of successful establishment and abundance of potential biological control agents adapted to specific climates (Julien & White, 1997; Paterson *et al*., 2014). Climate matching approaches are valuable in identifying regions in the native range that have similar climate conditions to the invaded distribution, which can save significant time and resources during surveys (Sutton, 2019). By using current occurrence records (GPS locations) of a species, climate-matching models are employed to quantify the correlation between the species and climate variables. These models are then used to simulate the potential distribution of the species in alternative geographic areas or under different climate conditions (Yuan *et al*., 2021). Consequently, this approach helps narrow down the search area for potential biological control agents, guiding practitioners in their selection process.

In this chapter, MaxEnt (Maximum Entropy Species Distribution Model Ver 3.4.1) (Phillips *et al*., 2006) models are mostly used as they constantly perform better than other ecological niche models (Elith *et al*., 2006; Wisz *et al*., 2008) and are amongst the most popular and widely used platforms (Merow *et al*., 2013). Since its introduction in 2006, MaxEnt has been cited in over 6000 publications (Phillips *et al*., 2017). It uses known occurrence records and environmental conditions to predict a species' potential unknown geographic range (Phillips *et al*., 2006). MaxEnt can use both presence and absence data but presence data are readily obtainable, whereas absence data are rarely available (Phillips *et al*., 2006). Absence data refers to locations that have been surveyed or sampled and where the species was not detected. These locations help the model understand the environmental conditions under which the species is likely not to be found. This chapter uses presence-only data exclusively.

When using MaxEnt it is advised to tune the parameters of the model to ensure the best possible model is created for the test species being considered (Phillips & Dudı´k, 2015). This may, however, be time-consuming and typically based on the performance of randomly selected portions of the dataset as training data (Phillips & Dudı´k, 2015). When dealing with biased or small datasets, the performance of the training data may not accurately reflect the model's predictive performance. As a result, the tuning process may be limited, and it is acceptable to rely on the default settings of the model (Phillips & Dudı´k, 2015). However, these default settings need to be validated across a wide range of environmental conditions, species, occurrences, and sample selection biases (Phillips & Dudı´k, 2015). The use of the default feature without optimization or validation seldom generates optimal outcomes (Fernández & Morales, 2019). Several settings affect the model's accuracy when using MaxEnt by determining the type and complexity of dependencies on the environment that MaxEnt tries to fit (Phillips & Dudı´k, 2015). For example, regularization parameters and feature types control the complexity of the model (Phillips & Dudı´k, 2015). These dependencies are known as features and are functions derived from environmental variables. The more complex the feature, the more complex the dependencies that are fit, however, more data may be needed (Phillips & Dudı´k, 2015).

Feature classes control the shape of the relationship between predictors and species presence/absence variables (i.e., the mathematical transformation of the predictor) and take the forms of either linear, hinge, quadratic, threshold, or product (Merow *et al*., 2013). Small sample sizes are most impacted by regularization. Therefore, model regularization should be tuned according to sample size (Elith *et al*., 2006). Feature classes serve as crucial inputs in the construction of the MaxEnt model, aiming to predict the probability distribution of suitable environmental conditions for the species. The selection and quality of these environmental variables significantly influence the accuracy and reliability of the MaxEnt model's predictions, providing insights into the ecological niche of the species across a specified geographic area size (Elith *et al*., 2006). Occurrence data used to calibrate the model can either be native and invaded range data combined or data points from the native range or invaded range on their own (Trethowan *et al*., 2011). It's likely that a combination of both the native and invaded range to calibrate the model will provide a better indication of a species likelihood of establishment or potential spread (Trethowan *et al*., 2011). As species are increasingly expanding their range by human-related dispersal or changes in their environment, such as climate change (Elith *et al*., 2010), this creates problems with species occurrence points no longer reflecting constant relationships with their environment and resulting in future scenarios where environmental combinations have not been effectively sampled (Elith *et al*., 2010). Range-shifting species, therefore, often violate the equilibrium assumption and need some degree of model extrapolation (Elith *et al*., 2010). Another reason for combining native and invaded ranges for this type of modelling is that introduced species are often found in areas that are different from their native range. The shift and establishment into a novel biome should be considered (Gallagher *et al*., 2010b). Created models are only as good as the occurrence data used. Errors in occurrence data introduce errors into the model that are difficult to account for, and therefore extra care must be taken to ensure the accuracy of every occurrence locality used in model development (Trethowan *et al*., 2011).

Pseudo-absence data play a crucial role in MaxEnt modelling by providing essential information about the background or absence conditions of a studied phenomenon (Phillips & Elith, 2010). In MaxEnt, pseudo-absence data represent the locations where the target species or event of interest is expected to be absent, based on available knowledge or assumptions (Lobo *et al*., 2010). The inclusion of pseudo-absence data in MaxEnt models is of paramount importance for several reasons. Firstly, pseudo-absence data help address the issue of sampling bias and spatial autocorrelation (Phillips & Elith, 2010). In many ecological and species distribution modelling studies, presence-only data are commonly available, while reliable absence data may be scarce or difficult to obtain. By incorporating pseudo-absence data, researchers can create a balanced dataset that accounts for the background conditions of the study area, reducing potential biases in model predictions. Secondly, pseudo-absence data assist in characterizing the environmental conditions where the target species is unlikely to occur (Lobo *et al*., 2010). By incorporating information about unsuitable habitats or areas with documented absence, MaxEnt models can learn to distinguish between suitable and unsuitable environmental conditions more accurately. This improves the model's ability to capture the true range of ecological requirements for the species or event being studied. Furthermore, pseudo-absence data help mitigate the problem of model overfitting (Phillips & Elith, 2010). Without proper representation of absence information, MaxEnt models may assign high probabilities to areas with no evidence of presence, leading to unrealistic predictions. By including pseudo-absence data, the model is encouraged to focus on the factors that genuinely contribute to the species' presence and absence, reducing the risk of overfitting and producing more reliable predictions. It is important to note that the selection and generation of pseudo-absence data require careful consideration to avoid introducing bias or inaccuracies into the modelling process (Liu, 2019b). The choice of appropriate sampling methods, ensuring representativeness of the background conditions, and considering spatial autocorrelation patterns are critical aspects when incorporating pseudo-absence data in MaxEnt models (Lobo *et al*., 2010; Phillips & Elith, 2010). Thus, pseudo-absence data are of great importance in MaxEnt modelling as they help address sampling bias, improve the model's ability to distinguish suitable from unsuitable habitats, and reduce the risk of overfitting (Phillips & Elith, 2010; Lobo *et al*., 2010).

In Chapter 2 it was found that foliar-feeding insects were the best candidates to start a management programme for the biological control of *R. pseudoacacia* in South Africa. In chapter 3 the potential insects as biological control agents were prioritized based on their potential to be successful biological control agents on *R. pseudoacacia* and a final three insects were selected for further study. These include the leaf galling midge, *Obolodiplosis robiniae*, *Macrosaccus robiniella*, a leaf mining microlepidopteran and the leaf mining beetle, *Odontota dorsalis*. Two of these insects, *O. robiniae* and *M. robiniella* have escaped their native range (North America) with their host and are established throughout Europe where *R. pseudoacacia* is found (Mally *et al*., 2021).

The aim of this study was to establish which of these potential biological control agents would be best suited to survive and thrive in South Africa based on climatic parameters from their native and invaded ranges.

4.2. Materials and Methods

4.2.1. MaxEnt models

MaxEnt models use a set of georeferenced species recorded locations as well as environmental parameters. Once the data had been imported, in this case from iNaturalist and the Global Biodiversity Information Facility (GBIF), the first step was to clean the data. This involved eliminating duplicate entries and rectifying coordinate inaccuracies (such as coordinates located in oceanic areas that are evidently implausible). Coordinates with less than optimal fine-scale precision were rounded to the nearest 0.5 °, unless they exhibited spatial errors surpassing 10 km as documented in the GBIF database. (Sutton, 2019) and were removed. For these models the following packages were used: “tidyverse”, “dismo”, “raster”, “corrplot”, “Hmisc”, “patchwork”, “ecospat”, “gridSVG”, “gridExtra”, “grid”, “ENMeval”, “spThin”, “viridis”, “viridisLite”, “mapdata”, “maptools”, “scales”, “geosphere”, “rgdal”, “rJava”, “rgeos”, “sp”, “sf”, “ggspatial”, “ecospat”, “rnaturalearth”, “rnaturalearthdata”.

For climatic or environmental parameters, Bioclimatic variables from WorldClim ver. 2 database (data available at: http://worldclim.org/version2) were used. These environmental predictors consist of a standard set of 19 climatic variables downloaded at 5 arc minute resolution. They consist of temperature and precipitation data spanning from 1950 to 2000 and are known to be predictive of species distributions (Gallagher *et al*., 2010a).These variables are indicated in table 4.1.

Table 4.1. The Bioclimatic variables used as environmental parameters in MaxEnt modelling are coded as follows from worldclim.org

Code	Variable
BIO1	Annual Mean Temperature
BIO2	Mean Diurnal Range
BIO3	Isothermality
BIO4	Temperature Seasonality
BIO5	Max Temperature of Warmest Month
BIO6	Min Temperature of Coldest Month
BIO7	Temperature Annual Range
BIO8	Mean Temperature of Wettest Quarter
BIO9	Mean Temperature of Driest Quarter
BIO10	Mean Temperature of Warmest Quarter
BIO11	Mean Temperature of Coldest Quarter
BIO12	Annual Precipitation
BIO13	Precipitation of Wettest Month
BIO14	Precipitation of Driest Month
BIO15	Precipitation Seasonality
BIO16	Precipitation of Wettest Quarter
BIO17	Precipitation of Driest Quarter

BIO18	Precipitation of Warmest Quarter
BIO19	Precipitation of Coldest Quarter

Background points and presence data: The analysis starts with the selection of occurrence records (presence data) for the species of interest. These records are used to define a cropped area. Background points (absence data) are then selected from this cropped area. The absence data represent locations where the species is known not to occur.

The cropped area surrounding the occurrence records is defined using Koppen-Geiger zones, which provide information about the climate and vegetation characteristics of different regions. These zones are downloaded from a specific source. Ecoregions where there are no occurrence records are eliminated from the analysis. This is done to ensure that the absence data are within a region where the target organism can potentially exist (Sutton, 2019).

Multicollinearity refers to the correlation between predictor variables used in the model. Strong correlations between predictors can make it difficult to identify the most meaningful predictor. To address this, highly correlated predictors (correlation coefficient above a specified threshold) are removed from the analysis. This helps in avoiding overfitting the models and inflated errors during extrapolation (Dormann *et al*., 2013).

Spatial autocorrelation refers to the tendency of nearby locations to have similar values. In the context of species distribution modelling, it means that the presence or absence of the species in one location may be related to its presence or absence in nearby locations. Spatial filtering is applied to thin or remove records that demonstrate spatial autocorrelation. This is done to prevent the influence of spatial patterns on the model output and to ensure the quality of the model (Boria *et al*., 2014; Veloz, 2009).

The model settings are tuned using the "ENMeval" package. This is done to strike a balance between the goodness-of-fit (how well the model fits the data) and the model complexity. Tuning the model settings is particularly important when dealing with spatially independent data or data with sampling bias. The package helps in selecting the ideal model settings by providing evaluation metrics, and the models with the lowest Akaike information criterion (AICc) value are considered the best fit models (Galante *et al*., 2018; Muscarella *et al*., 2014; Sutton, 2019). To estimate model overfitting, the 10th percentile calibration omission rate (OR10) is applied. This rate identifies the prediction threshold from the model with the lowest prediction values that excludes 10% of the calibration data. Higher suitability points above this threshold are considered as potential outliers. Additionally, the "threshold-independent area under the curve (AUC) of receiver operating characteristics (ROC)" is used to validate the MaxEnt results. AUC values range from 0.5 to 1.0, with higher values indicating better model performance (Fielding & Bell, 1997; Mousazade *et al*., 2019).

Multivariate environmental similarity surface (MESS) evaluation: MESS maps are evaluated to assess whether the models are interpolating (predicting within the range of calibration data) or extrapolating (predicting outside the range of calibration data). Negative MESS values indicate potential extrapolation and can have negative consequences on model performance, especially when predicting species into novel regions (Elith *et al*., 2010; Radomski *et al*., 2022).

4.2.2. CLIMEX models

Additional models were created using CLIMEX (Version 4) to examine the potential establishment of three prioritized biological control agents. *Obolodiplosis robiniae* was used as an example in this regard. This was achieved through a regional climate match method, as described by Sutherst *et al*. (2018). CLIMEX uses known climates for a species to infer their climatic preferences and adjusts relevant parameters until the model's results align with the species' known distributions. These parameter settings can then be used to estimate the species' distribution in other areas based on climate (Sutherst *et al*., 2018).

The species' parameters summarize their responses to climate factors that constrain their geographical distribution. Three groups of variables limit the growth and survival of the species. The "Weekly" and "Annual" indices represent the suitable temperature range (Temperature Index, TI) and moisture range (Moisture Index, MI) for growth and reproduction. Stress indices, including hot stress (HS), cold stress

(CS), wet stress (WS), and dry stress (DS), indicate the extremes of temperature and moisture within which the species can survive. Some species may have additional limiting conditions such as dormancy or diapause (for insects), or heat accumulation requirements per generation (not considered in this study) (Sutherst *et al*., 2018).

The Ecoclimate Index (EI), derived from the growth and stress indices, provides an overall assessment of the species' suitability for long-term survival and growth. Constraints such as the growing season and diapause are taken into account in determining the EI value (Sutherst *et al*., 2015).

For this study, the "Match Climates, Regional" (MCR) application, an extension of CLIMEX, was used. It simultaneously runs the application for locations projected across geographic space, combining the results to retain the best outcome for each region (Sutherst *et al*., 2015).

The distribution of *Pseudoacacia* from South Africa was projected onto the native range (USA) and invaded range (Europe) of these insects, where they have established and are found in high infestations on the target plant. Data from GBIF and iNaturalist were used, and comparisons between the native and invaded ranges were based on standard variables defined by CLIMEX, including monthly rainfall, monthly temperature, and default weights of one. Additional information on monthly temperature and rainfall records was obtained, and historical data sets from CliMond (CM10_1975H_V1) were used for the model runs (Kriticos *et al*., 2015).

The MCR function is favoured by biological control practitioners as it allows for the refinement of search areas for biological control agents and the identification of regions with climatic similarity to the release region. It is suggested by Sutherst *et al*. (2015) that matched areas are likely to support the establishment of biological control agents.

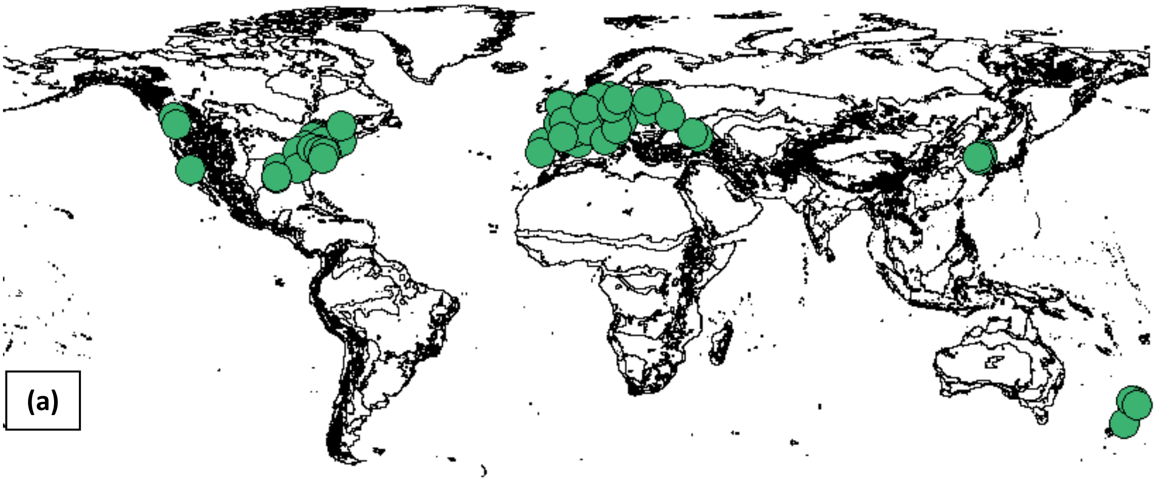
To visualize the results, Geographical Information System (GIS) software ARCMAP 10.8.1 was used to convert the findings into raster files and create heat maps. They represent the predicted habitat suitability or probability of occurrence for a species across a geographic area. These maps visually depict the model's estimation of how suitable different locations are for the species based on the environmental variables provided to the model. Heat maps are typically presented as colour gradients, where different colours represent different levels of habitat suitability. For example, warmer colours (e.g., red or orange) may indicate areas with higher predicted suitability, while cooler colours (e.g., blue or green) may represent lower suitability.

4.3. Results

4.3.1. MaxEnt Models for the three potential biological control agents

4.3.1.1. *Obolodiplosis robiniae*

The native range for *O. robiniae*, figure 4.1 (a), is North America but it has spread with its host and has invading mostly Europe, but also parts of Asia and more recently New Zealand (Mally *et al*., 2021). After cleaning, removing duplicate records and spatial thinning, the amount of occurrence records for *O. robiniae* went down from 322 records to 285 to be used in model calibration. Figure 4.1 (b) shows all the occurrence data points overlaid onto the Koppen-Geiger layer and the eco-regions to which they belong.



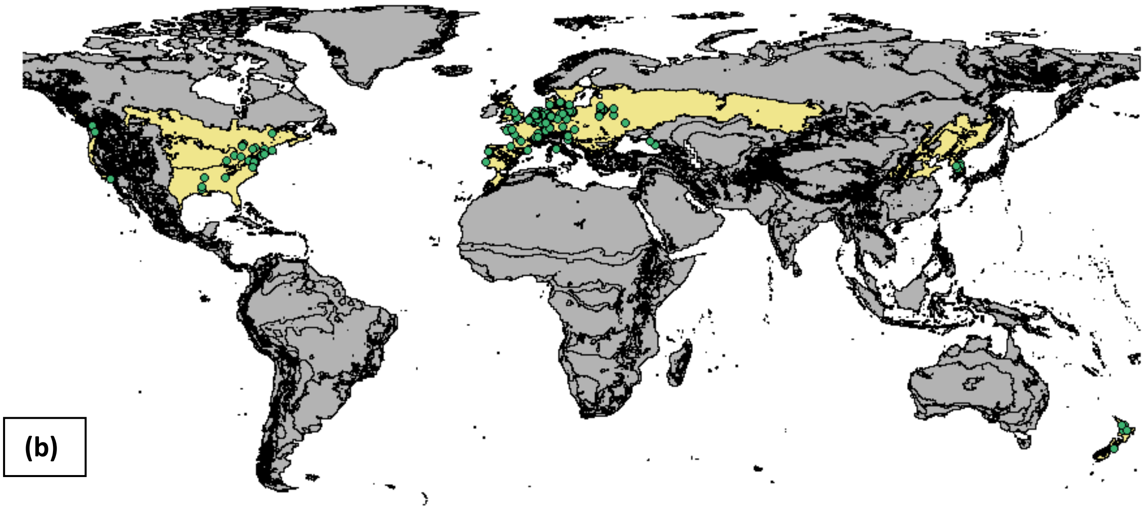
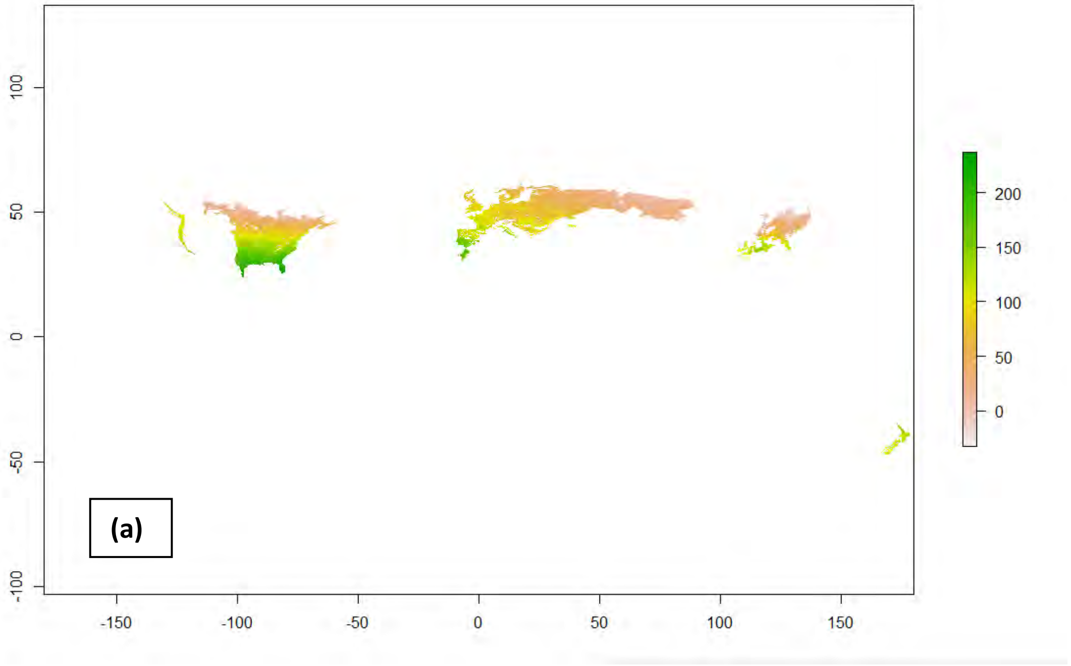


Figure 4.1. (a). *Obolodiplosis robiniae* location records visualised by distinct green dots overlayed onto the Koppen-Geiger layer seen in the USA, Europe, Asia and New Zealand. (b) The specific ecoregions in which *O. robiniae* locations records are found, also indicated by fine green dots as specific loacations.

Background data points were selected from these selected zones because the species is most likely to be found in the same eco-region. The Worldclim layers were masked to these zones shown in figure 4.2.(a) and background points were then generated in these isolated regions, figure 4.2.(b).



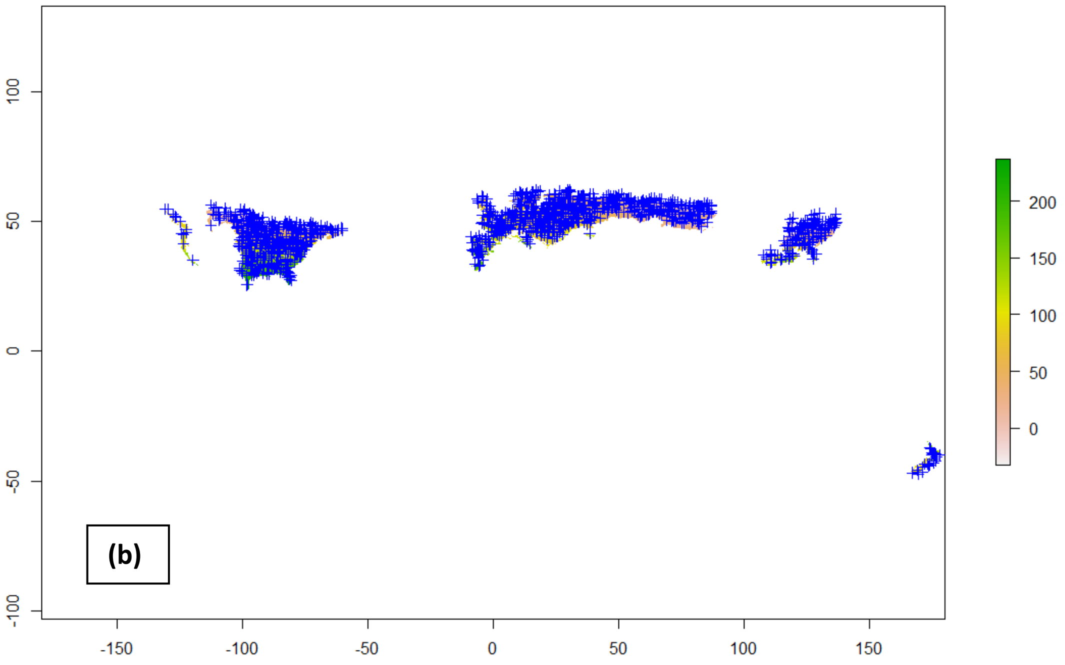


Figure 4.2. (a) Worldclim layers masked to only keep the selected zones from the Koppen-Geiger map. (b) Background data points plotted over the same Koppen-Geiger zones where *Obolodiplosis robiniae* occurrence locations are (are marked with blue crosses). The colour gradient in the legend refers to the suitability of the cliamatic layers.

The following graph indicates layers that contributed the most to the model; the other layers were removed to reduce multicollinearity between environmental predictors (Figure 4.3.).

Figure 4.3. Pairwise correlations between the selected bioclimatic variables. BIO1, BIO2, BIO3, BIO4, BIO8 and BIO12 were selected as they were under the correlation co-efficient of 0.70 (r |>0.70|).

For optimal models, the following was considered. The AUC (Area Under the Curve) graph for MaxEnt provides insights into the performance of a MaxEnt classifier across different feature classes. From figure 4.4. the MaxEnt classifier performs very well for the "H" feature class, achieving a high initial AUC score of 0.96, which suggests excellent discrimination capability. Similar to the "H" feature class, the MaxEnt classifier performs exceptionally well for the "HLQ" feature class, as indicated by the initial AUC score of 0.96. The subsequent AUC score of 0.95 suggests that the classifier maintains a strong discriminatory power for this feature class, remaining consistently effective. The MaxEnt classifier performs well initially for the "HLQPT" feature class, with an AUC score of 0.94. The subsequent AUC score of 0.95 indicates that the classifier's performance remains relatively stable and high for this feature class, similar to the "H" and "HLQ" feature classes. Overall, the AUC graph for MaxEnt demonstrates that the classifier performs very well for all three feature classes, consistently achieving high AUC scores. The slight differences in the AUC scores across feature classes may indicate varying levels of discriminative power among them.

The omission rate in figure 4.4. provides information about the performance of the classifier in terms of the rate at which it incorrectly omits or fails to classify occurrences correctly. When the regularization multiplier is set to 2.5, the classifier's performance improves, leading to a lower and stable omission rate of 0.10, indicating that it correctly classifies around 90% of instances. The stabilization occurs when the regularization multiplier is set to 5.0. Initially, the MaxEnt classifier has a higher omission rate of 0.13 for the "HLQPT" feature class, suggesting a higher proportion of incorrect classifications or omissions. However, when the regularization multiplier is set to 5.0, the classifier's performance improves significantly, leading to a stable omission rate of 0.10, indicating a correct classification rate of around 90%. Thus, the omission rate graph demonstrates the classifier's performance for each feature class at different regularization multiplier values. It indicates that increasing the regularization multiplier generally improves the classifier's performance by reducing the omission rates. The stabilization at an omission rate of 0.10 suggests that, with the respective regularization multiplier values, the MaxEnt classifier achieves a stable and relatively high rate of correct classifications, correctly classifying around 90% of instances for all feature classes.

The delta AICc in figure 4.4. provides insights into the relative model performance and the trade-off between model complexity and fit. A decrease in the delta AICc indicates improved model performance relative to the reference model. The higher delta AICc values indicate poorer performance compared to the reference model. However, as the regularization multiplier increases within the range specified, the model's performance improves, leading to a decrease in the delta AICc, ultimately reaching 0. Therefore, the delta AICc graph for MaxEnt illustrates the model performance for each feature class at different regularization multiplier values. The regularization multiplier values at which the delta AICc reaches 0 signify the optimal regularization parameter values for each feature class, indicating the best-fit models.

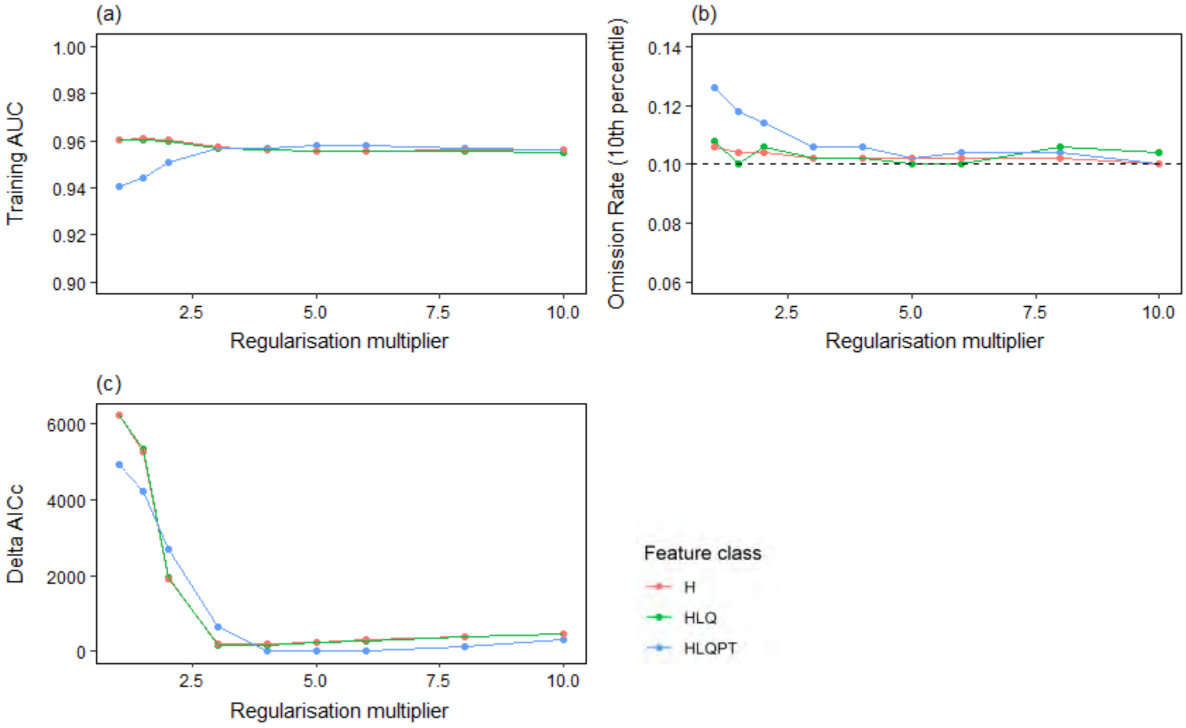
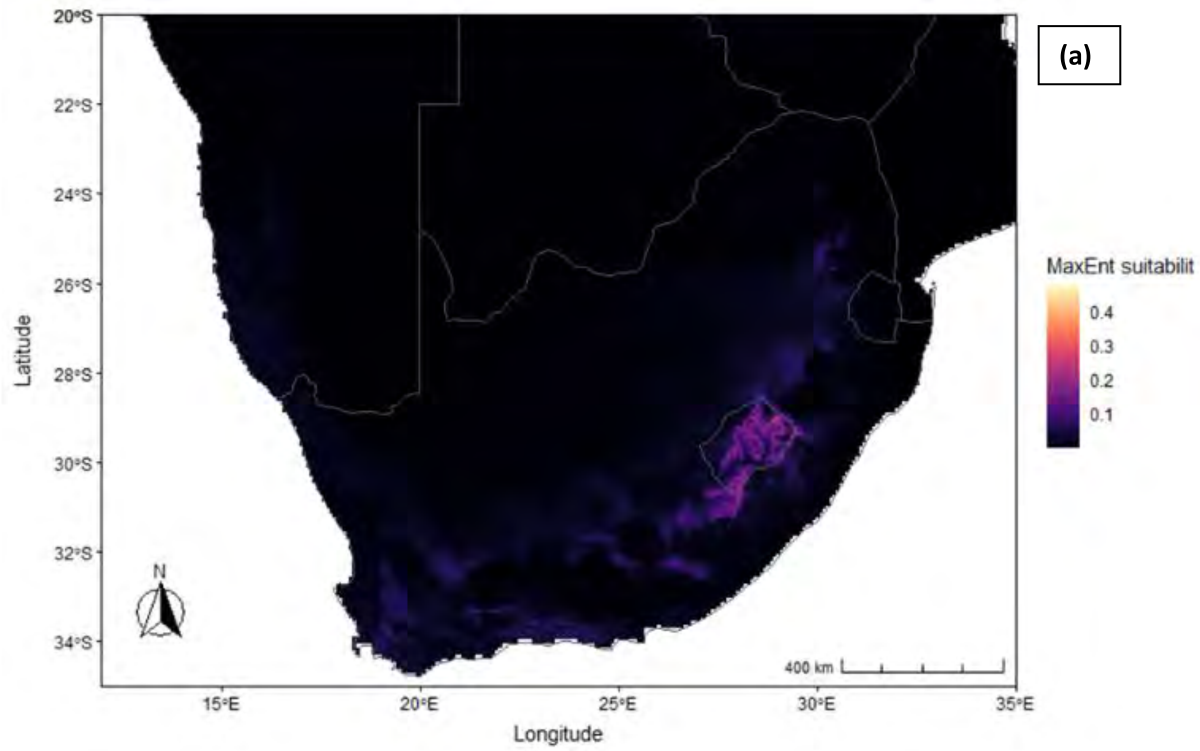


Figure 4.4. Model tuning in MaxEnt for *Obolodiplosis robiniae* based on occurrence data in the native (USA) and invaded (Europe) range. (a) AUC: prediction of species occupancy potential. (b) OR_{10}: model overfitting by a binary threshold that excludes values of 10% of the calibration occurrences from the model with low values of prediction or where it has a predicted omission rate of 0.10. (c) Delta AICc: to establish complexity and optimal model settings, the smaller the value the better the model fit. Here feature class combinations were considered ("HLQPT" represents: "hinge," "linear," "quadratic," "product" and "threshold") as well as regularization multipliers (0.5 – 10.0).

The resulting maps generated by MaxEnt is a continuous surface reflecting the probability or suitability of environmental conditions for the target species across a geographic area. The results are commonly visualized through heat maps, which employ a color gradient to represent the varying probabilities. Warmer colors indicate higher suitability, while cooler colours suggest lower probability. The map for *O. robiniae* suggests that the most suitable areas for release would be along the Drakenberg escarpment but especially within and around Lesotho's borders.

In terms of interpolation and extrapolation, MESS Maps (Figure 4.5.b) resulting from the MaxEnt models show distinct methods for the estimation or prediction of values at locations lacking direct data observations. Interpolation pertains to the estimation of values within the established range of observed data points. Within MaxEnt maps, this method serves the purpose of predicting the probability or distribution of a variable between sampled points. While extrapolation involves the prediction of values beyond the scope of observed data points. In MaxEnt maps, extrapolation is applied to forecast the probability or distribution of a variable in areas devoid of data points. Extrapolation poses heightened challenges, necessitating assumptions about the variable's behavior beyond the observed range, consequently introducing increased uncertainty. Extrapolation can be seen for the prediction model run for *O. robiniae.*



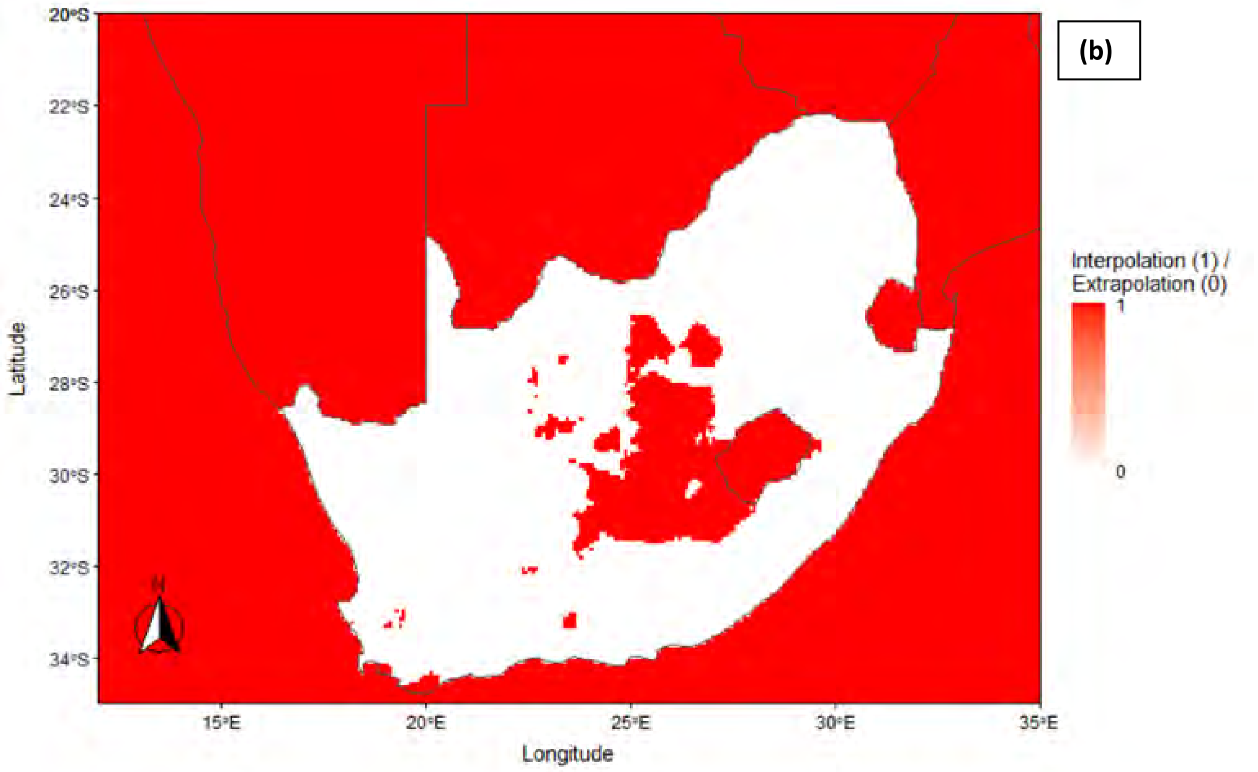


Figure 4.5. The climate matching maps for *Obolodiplosis robiniae* providing information for: (a) climatic suitability in South Africa, where models were calibrated using occurrences from the species' native and invaded ranges. (b) The MESS (Multivariate Environmental Similarity Surface) is used to indicate areas where MaxEnt was extrapolating (denoted as MESS-) and areas where it was interpolating (denoted as MESS+).

4.3.1.2. *Macrosaccus robiniella*

The native distribution in figure 4.6 (a) of *M. robiniella* is also North America, but it has expanded its range by invading into Europe with its host. After data cleaning and removal of duplicate records, the number of occurrence records available for model calibration decreased from 794 to 578. Along with the occurrence data, the Koppen-Geiger layer was loaded to generate background points representing absence data. Figure 4.6 (b) displays all occurrence data points superimposed on the Koppen-Geiger layer, indicating the eco-regions to which they belong.

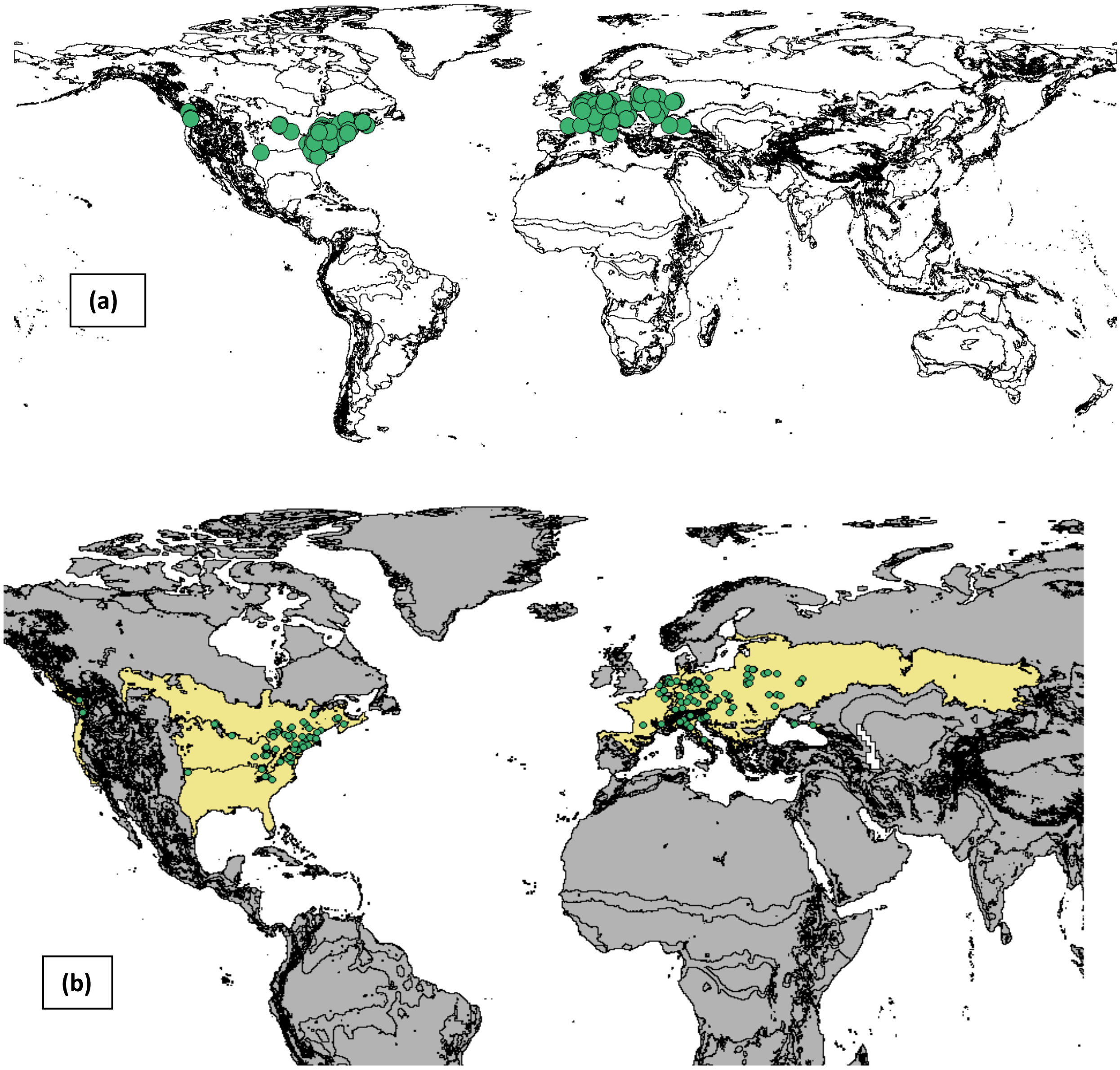


Figure 4.6. (a) *Macrosaccus robiniella* location records visualised by distinct green dots overlayed onto the Koppen-Geiger layer. (b) The specific ecoregions within which *M. robiniella*locations records are found (small green dots) indicated as the yellow region.

Background data points were selected from these selected zones because the species is most likely to be found in the same eco-region. The Worldclim layers were masked to these zones shown in figure 4.7.(a) and background points were then generated in these isolated regions, figure 4.7.(b).

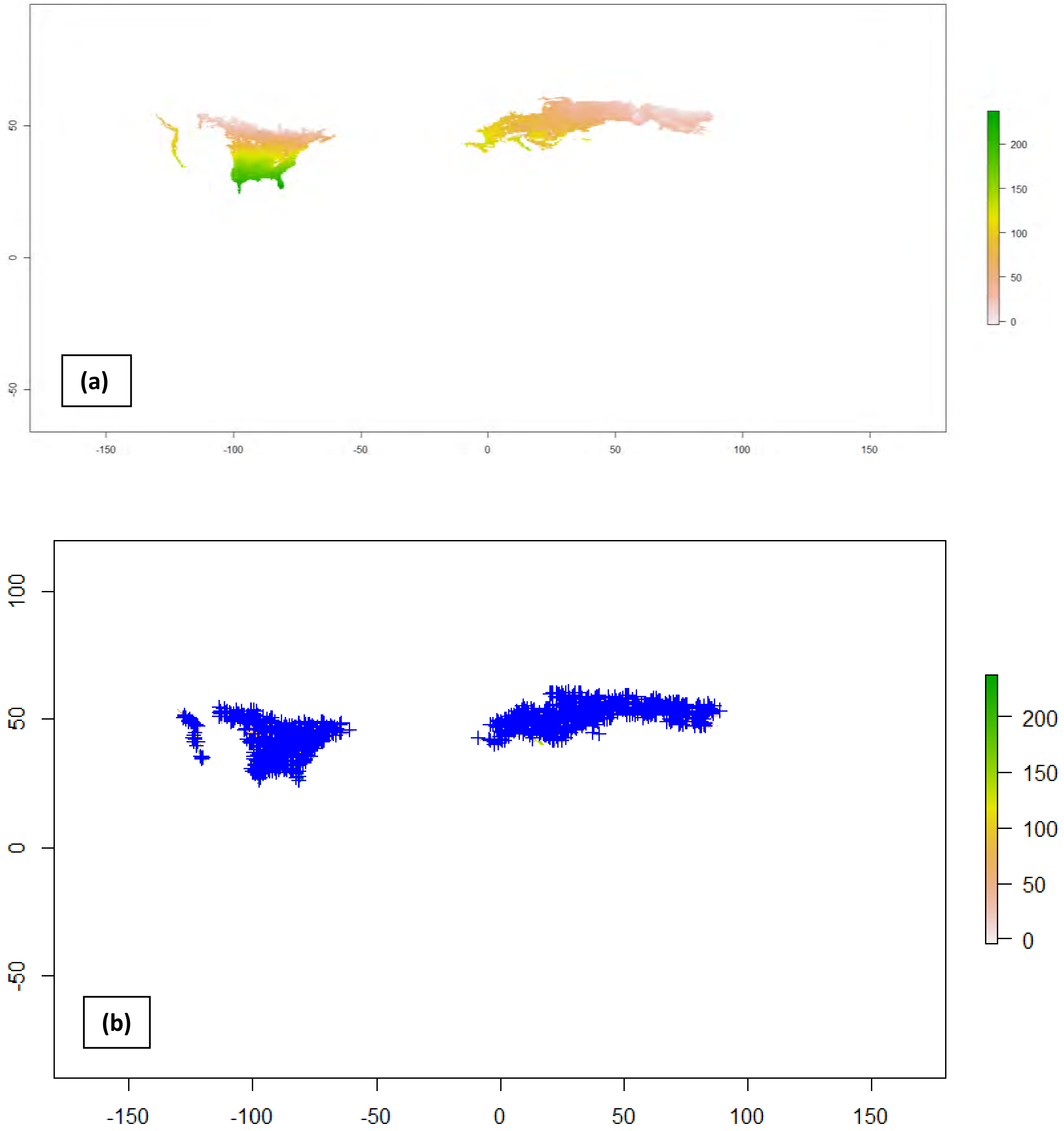


Figure 4.7. (a) Worldclim layers masked to keep only the selected zones from the Koppen-Geiger map. (b) Background data points plotted maked by blue crosses over the same Koppen-Geiger zones where *Macrosaccus robiniella* occurrence points are.

To prevent overfit models and identify the most meaningful predictors, multicollinearity between environmental layers was reduced with the correlation co-efficient (r |>0.70|). For this model environmental predictors BIO1, BIO3, BIO4, BIO8, BIO9 and BIO12 were used (Figure 4.8.).

Figure 4.8. Pairwise correlations between the selected bioclimatic variables. BIO1, BIO3, BIO4, BIO8, BIO9 and BIO12 were selected as they were under the correlation co-efficient of 0.70 (r |>0.70|) which suggests a strong relationship between predicted and observed values, indicating good model fit, with factors such as feature relevance, data quality, model tuning, sample size, and spatial autocorrelation potentially influencing the result.

The ensuing factors contributed to determining the most suitable model parameters for *M. robiniella*, as illustrated in Figure 4.4. The AUC scores within the "H" and "HLQ" feature classes reveal a consistent and strong performance of the MaxEnt classifier, maintaining AUC scores within the 0.93-

0.94 range for both categories. This consistency underscores the MaxEnt classifier's effectiveness in these feature classes. The stability of the AUC scores suggests that the classifier maintains a steady discrimination capability for positive and negative instances related to these feature classes throughout the training process. Initial performance of the MaxEnt classifier for the "HLQPT" feature class is slightly lower at 0.93. However, as the training progresses and the regularization multiplier increases, the AUC score improves, reaching 0.94, which indicates better discrimination capability. Eventually, the AUC score stabilizes at the same level as the other feature classes.

The omission rate for figure 4.9 illustrates the classifier's performance in terms of the rate of incorrect classifications or omissions for different feature classes. The "H" feature class shows a relatively stable performance, staying closest to the desired omission rate of 0.10. The "HLQ" feature class improves gradually but may still have room for further refinement to reach the desired rate. However, the "HLQPT" feature class exhibits a higher omission rate, indicating a greater challenge for the classifier to correctly classify instances related to this feature class.

The delta AICc value for the "HLQPT" feature class has a high initial delta AICc value, suggesting that the initial model for the "HLQPT" feature class has poorer performance than the reference model. However, as the regularization multiplier increases, the model's performance improves, leading to a decrease in the delta AICc and ultimately reaching 0. Achieving a delta AICc value of 0 indicates that the model utilizing the chosen regularization multiplier offers the optimal data fit for the "HLQPT" feature class. However, for the "HLQ" and "H" feature classes, a decrease in AICc value might not necessarily result in the best data fit compared to the "HLQPT" feature class, since their values do not reach 0.

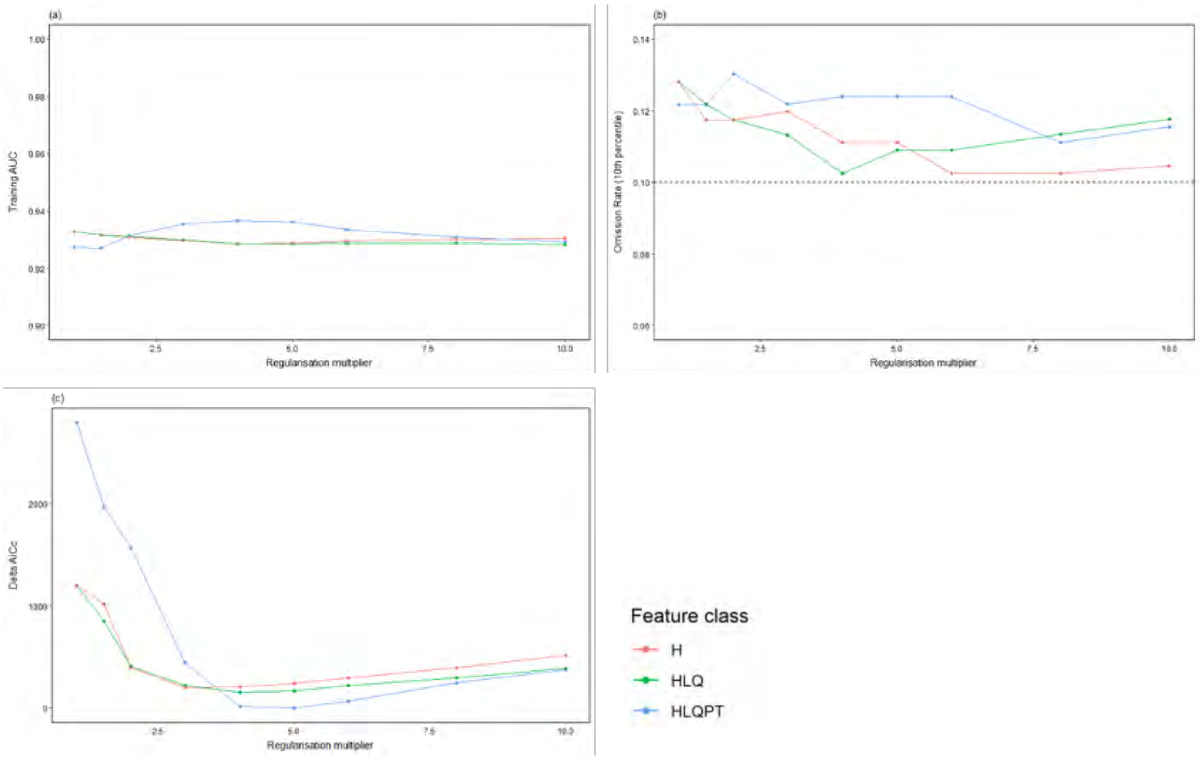


Figure 4.9. Model tuning experiments for *Macrosaccus robiniella*, for native and invaded-range models combined. See Figure 4.4. for details.

The predictive potential for the establishment of *M. robiniella* (figure 4.10 a) is slightly larger than *O. robiniae* in South Africa, also along the range of the Drakensberg Mountains in the country and around Lesotho, where the tree is also abundant. Unfortunately, this still does not cover the entire range of *R. pseudoacacia* in South Africa. The MESS map (figure 4.10 b) for *M. robiniella* suitability in South Africa indicated that interpolation space included Lesotho and areas south and south-west from there. Extrapolation may have an influence in the model.

In MaxEnt maps, extrapolation is utilized to predict the probability or distribution of a variable in regions where no data points are available. Extrapolation presents elevated difficulties, requiring assumptions about the variable's behaviour beyond the observed range, thereby introducing heightened uncertainty. Extrapolation is evident in the prediction model for *M. robiniella*. Similar to the preceding model, this one also exhibits slight extrapolation, potentially impacting its predictability.

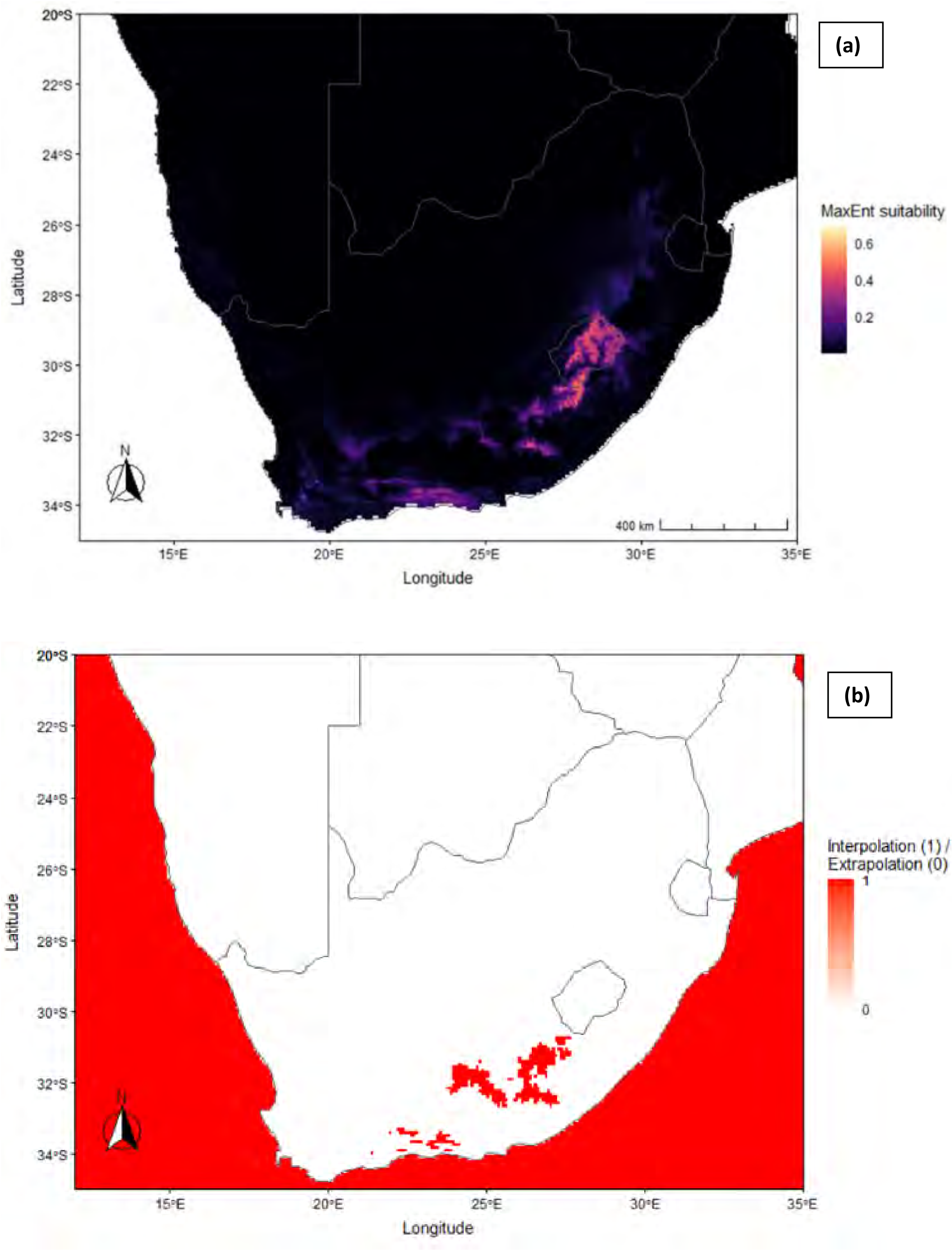


Figure 4.10. The climate matching maps for *Macrosaccus robiniella* providing information for: (a) climatic suitability in South Africa, where models were calibrated using occurrences from the species' native and invaded ranges. (b) The MESS (Multivariate Environmental Similarity Surface) is used to

indicate areas where MaxEnt was extrapolating (denoted as MESS-) and areas where it was interpolating (denoted as MESS+). This helps identify the regions where the model's predictions were outside the range of observed data (extrapolation) and where they were within the range (interpolation).

4.3.1.3. *Odontota dorsalis*

The original range of *O. dorsalis* is also confined to North America, and unlike the first two insects mentioned, it has not extended beyond its native distribution as illustrated in figure 4.11 (a). Following the data cleaning process and removal of duplicate records, the number of occurrence records available for model calibration reduced from 286 to 112. In addition to the occurrence data, the Koppen-Geiger layer was used to generate background points representing the absence of the species. Figure 4.6 (b) illustrates the overlay of all occurrence data points onto the Koppen-Geiger layer, providing insights into the eco-regions to which they are associated.

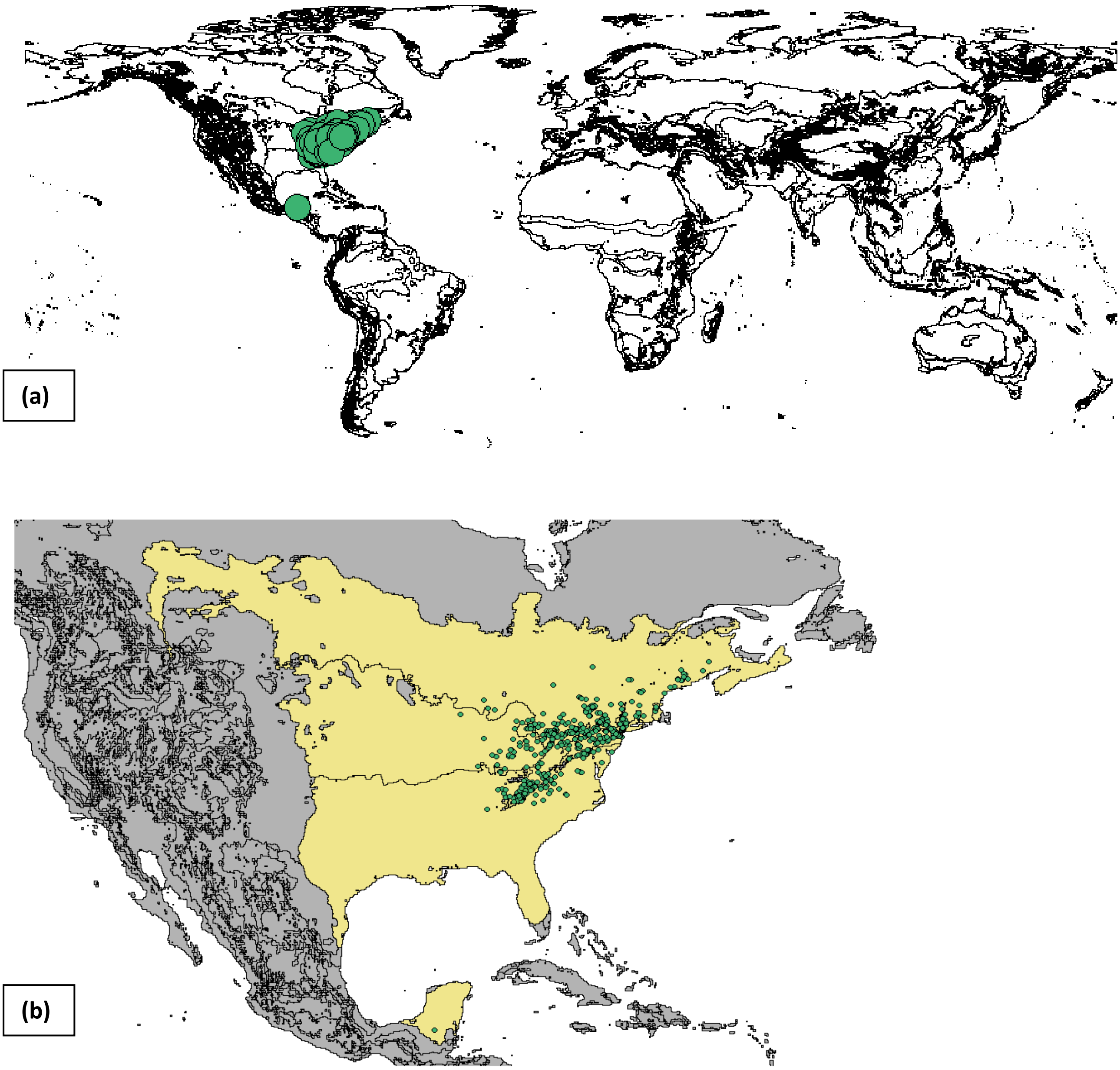


Figure 4.11. (a) *Odontota dorsalis* location records visualised by distinct green dots overlayed onto the Koppen-Geiger layer. (b) The specific ecoregions within which *Odontota dorsalis* locations records are found (small green dots) indicated as the yellow region.

Background data points were selected from these selected zones because the species is most likely to be found in the same eco-region. The Worldclim layers were masked to these zones shown in figure 4.12. (a) and background points were then generated in these isolated regions, figure 4.12. (b).

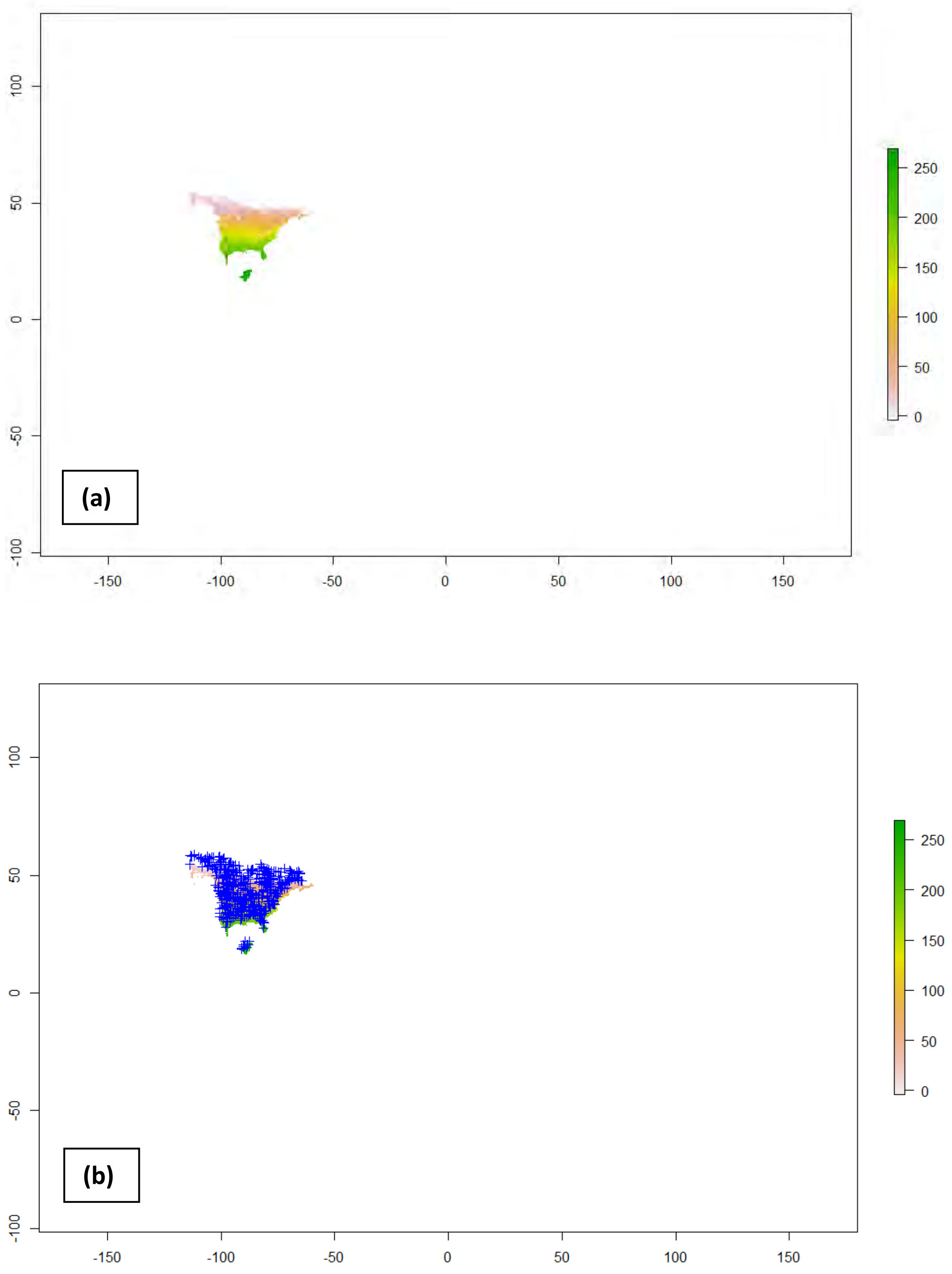


Figure 4.12. (a) Worldclim layers masked to keep only the selected zones from the Koppen-Geiger map. (b) Background data points plotted over the same Koppen-Geiger zones where *Odontota dorsalis* occurrence points are.

To prevent overfit models and identify the most meaningful predictors, multicollinearity between environmental layers was reduced with the correlation co-efficient (r |>0.70|). For this model environmental predictors BIO1, BIO2, BIO8, BIO9 and BIO12 were used (Figure 4.13).

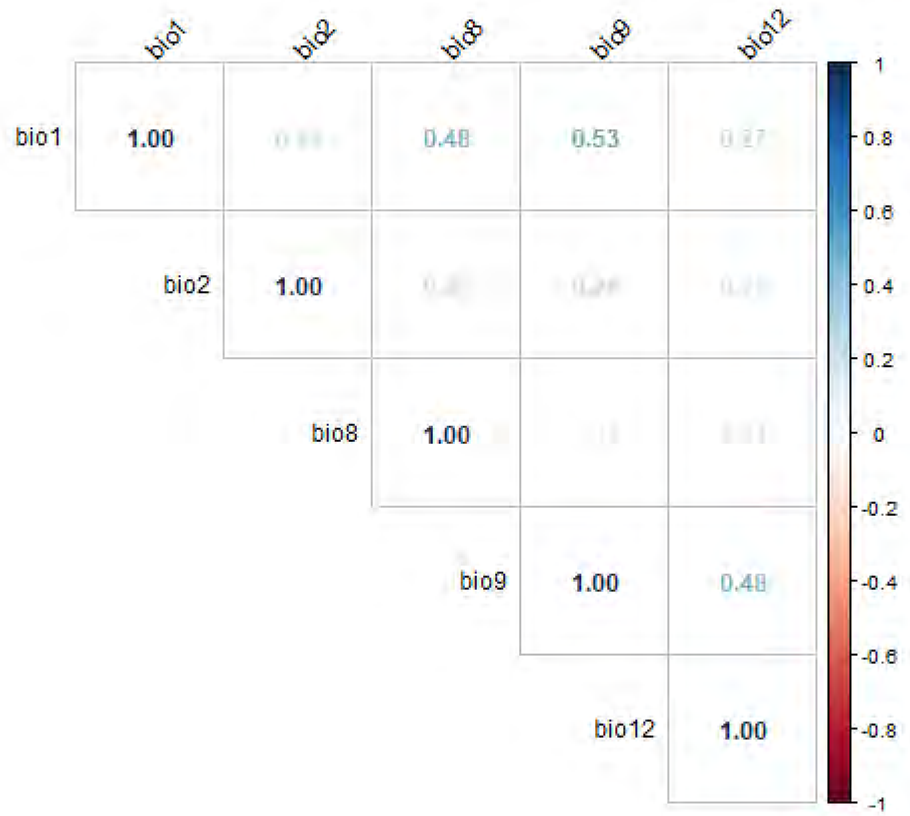


Figure 4.13. Pairwise correlations between the selected bioclimatic variables. BIO1, BIO2, BIO8, BIO9 and BIO12 were selected as they were under the correlation co-efficient of 0.70 (r |>0.70|).

In figure 4.14 the AUC score indicates that the MaxEnt classifier initially performs well for the "HLQPT" feature class, achieving a high AUC score just above 0.94. The decreasing trend in the AUC score suggests a decline in the classifier's discrimination capability for the "HLQPT" feature class as the regularization multiplier increases. The decreasing trend in the AUC scores suggests a slight decline in the discrimination capability of the classifier for both the "H" and "HLQ" feature classes as the regularization multiplier increases. Thus, the AUC graph for MaxEnt illustrates the performance of the classifier for each feature class as the regularization multiplier varies. The "HLQPT" feature class shows a steeper decrease in the AUC score, indicating a more significant decline in the discrimination capability of the classifier as the regularization multiplier increases. The "H" and "HLQ" feature classes also experience a gradual decline in AUC scores but exhibit a more stable performance than the "HLQPT" feature class.

In figure 4.14 the omission rate for the "HLQ" feature class dips below 0.10 at a regularization multiplier of about 1, indicating that the classifier is correctly classifying more than 90% of instances for this particular feature class. However, it increases again and nearly reaches 0.10 at a regularization multiplier of 5.0, suggesting a slight deterioration in performance compared to the initial dip. The erratic behaviour of the omission rate for the "HLQ" feature class indicates fluctuations in the classifier's ability to correctly classify instances, resulting in an inconsistent omission rate. The erratic behaviour of the omission rate for the "H" feature class indicates fluctuations in the classifier's ability to correctly classify instances, resulting in an inconsistent omission rate similar to the "HLQ" feature class. The omission rate for the "HLQPT" feature class does not reach the 0.10 threshold and decreases further than 0.11. This suggests that the classifier struggles to achieve a low omission rate for the "HLQPT" feature class. The decreasing trend beyond 0.11 indicates a relatively higher proportion of incorrect classifications or omissions for this feature class compared to the other feature classes.

The delta AICc value for the "H" feature class (figure 4.14) indicates that the model for the "H" feature class has a higher AICc value than the reference model, suggesting poorer performance. As the regularization multiplier increases, the model's performance improves, leading to a decrease in the delta AICc. Attaining a delta AICc value of 0 at a regularization multiplier of 6 implies that the model employing this specific regularization multiplier offers the optimal data fit for the "H" feature class. As the regularization multiplier undergoes variation, the performance of the model differs for each feature class. Notably, the "H" feature class displays the most substantial enhancement, as evidenced by the reduction in delta AICc to 0, signifying the best data fit. The "HLQ" and "HLQPT" feature classes also experience improvements, but their delta AICc values level out at higher levels compared to the "H" feature class, indicating relatively less optimal model performance.

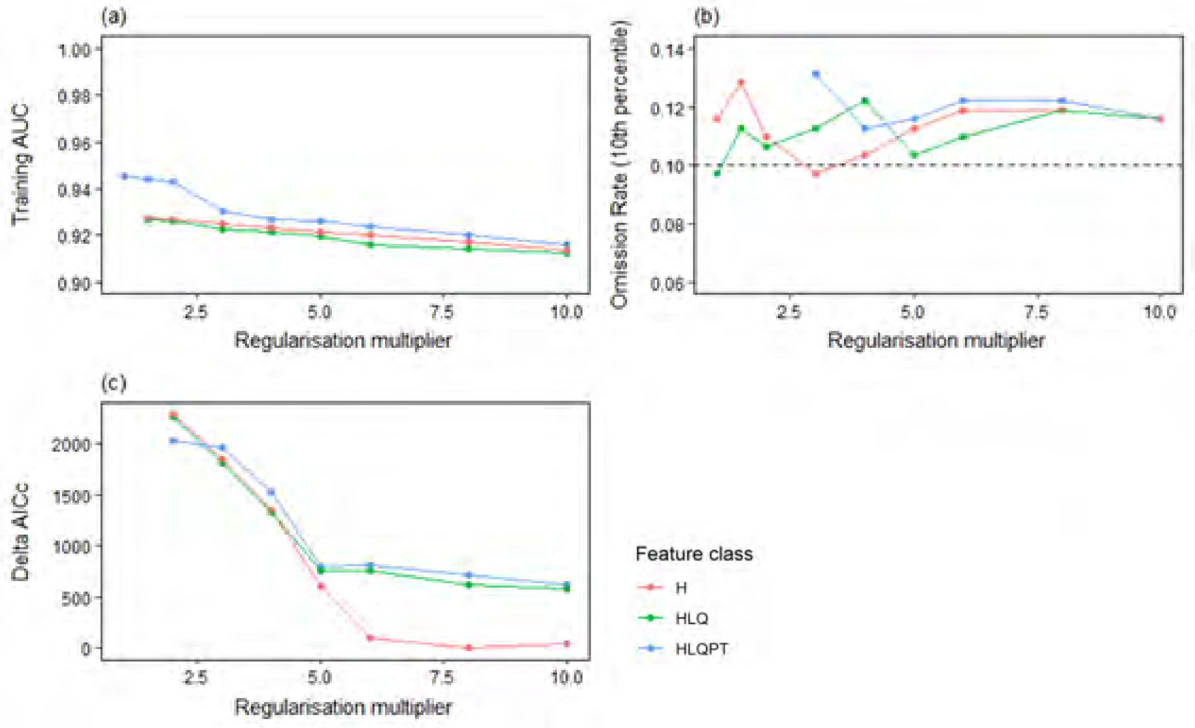


Figure 4.14. Model tuning experiments for *Odontota dorsalis*, for native and invaded-range models combined. See Figure 4.4. for details.

Odontota dorsalis has the best climate suitability for establishment in South Africa based on climatic variables from the three insects modelled in this study (figure 4.15a). It is most likely to establish north and east of Lesotho, as well as within Lesotho, but also has the potential to establish along the Drakensberg Mountain range along the eastern side of South Africa. It has higher suitability areas in the Western Cape and Eastern Cape than the other two insects but also has higher suitability in the most northern parts of South Africa in the Limpopo Province. Slight suitability is predicted in central South Africa, which was not seen from the other two models, but it has no prediction suitability in the Northern Cape Province, which is seen from the previous models as well. The MESS map for *O. dontota* (figure 4.15 b) shows interpolation in the same areas as the highest predicted suitability areas for establishment of this insect. Extrapolation is therefore unlikely to be a significant influence on this model.

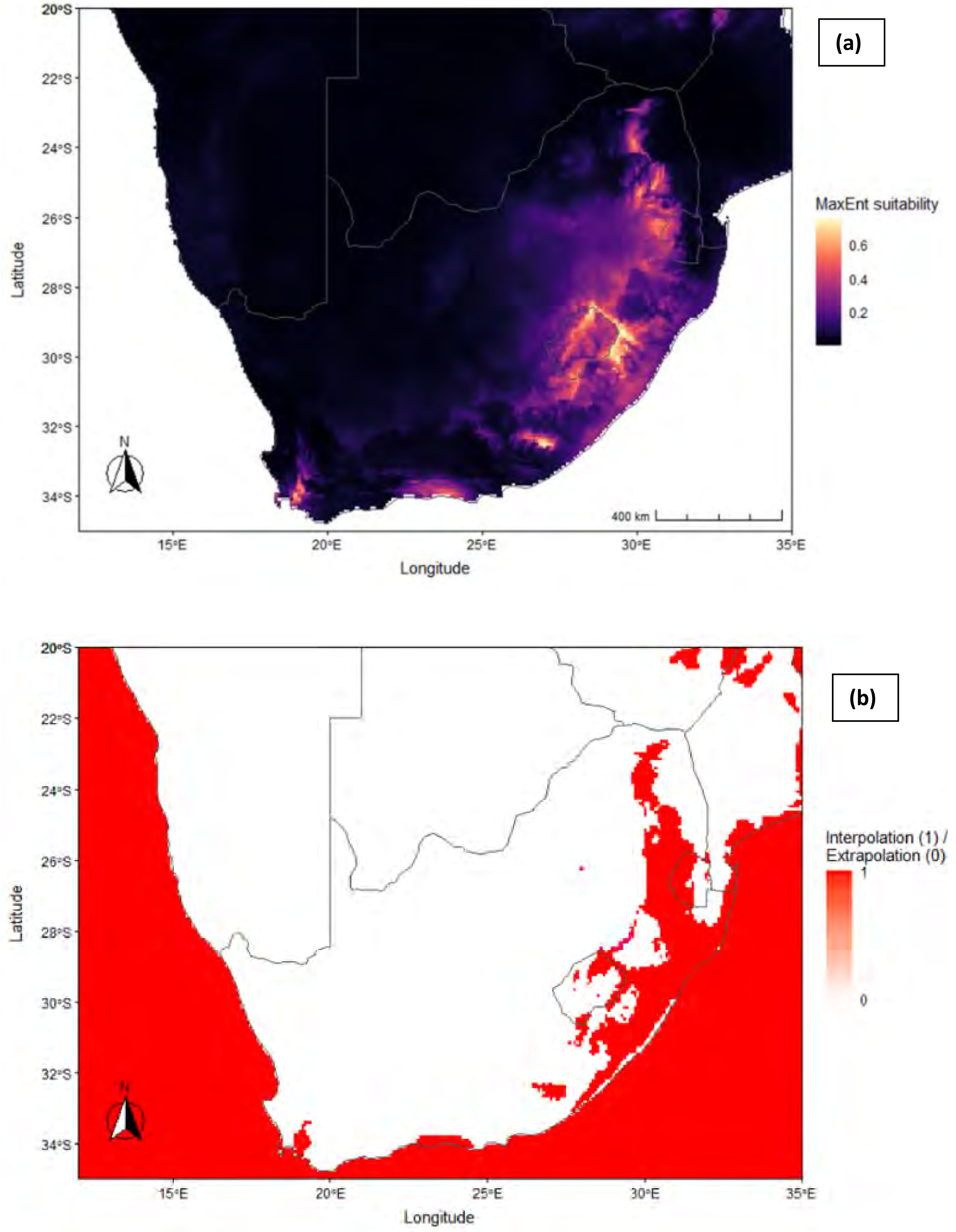


Figure 4.15. The climate matching maps for *Odontota dorsalis* providing information for: (a) climatic suitability in South Africa, where models were calibrated using occurrences from the species' native ranges (this species has not escaped its native range). (b) The MESS (Multivariate Environmental Similarity Surface) is used to indicate areas where MaxEnt was extrapolating (denoted as MESS-) and

areas where it was interpolating (denoted as MESS+). This helps identify the regions where the model's predictions were outside the range of observed data (extrapolation) and where they were within the range (interpolation).

4.3.2. CLIMEX model for *O. robiniae*

CLIMEX models are usually used to predict the spread of invasive species (Sutherst *et al*., 1999) but in this case they are used to determine whether a biological control agent will have the potential to establish in a novel environment where it is purposefully released.

As South Africa’s climate was back projected onto the native range (North America) and invaded range (Europe) figures 4.16 show that the areas where the insects are found in their native range (North America) have a similar environment to South Africa based on climatic variables used. This is indicated visually with a light red shape on the heat map indicating suitability. The invasive range (Europe) in areas where the occurrence records are found has an even more similar climate to South Africa. This is indicated with a dark red shade indicating highly similar climate in that area layered onto the map of Europe (figure 4.16 b).

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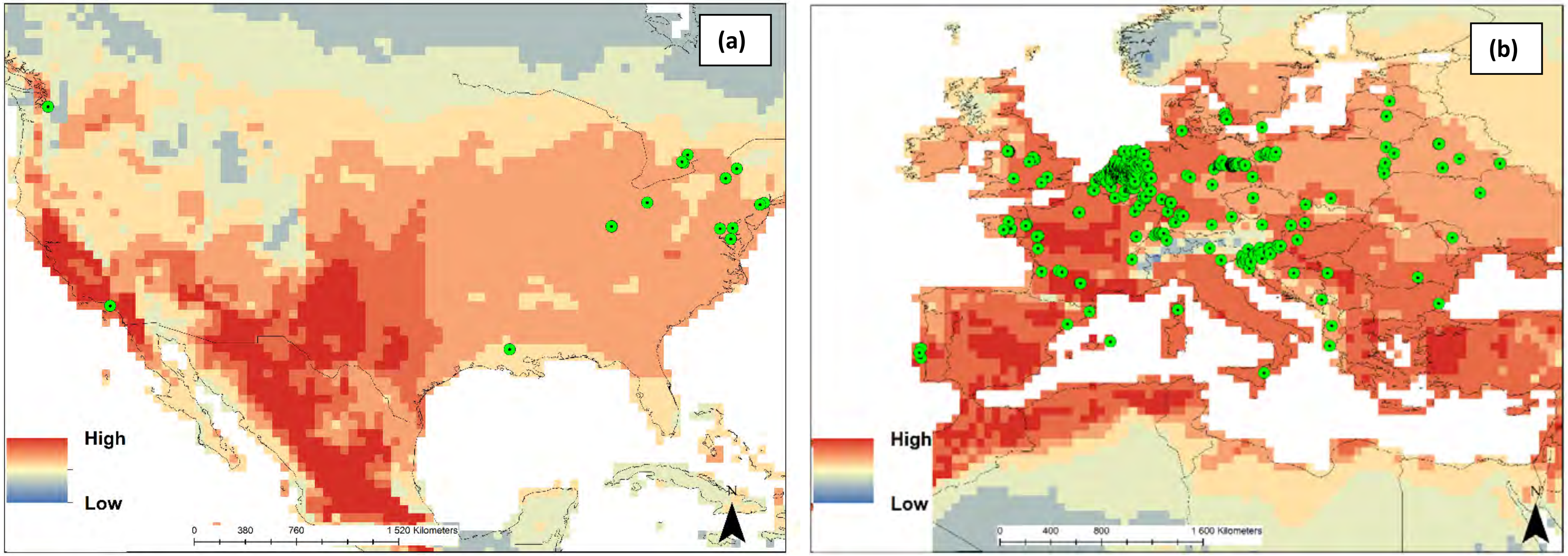


Figure 4.16. (a)The native range of *Obolodiplosis robiniae* (North America) with occurrence records (sourced from GBIF and iNaturalist) indicated with green dots modelled with climatic variables from South Africa. Warmer colours indicate high similarity between North America and South Africa while cooler colours are less similar. (b) The invaded range of *O. robiniae* (Europe) with occurrence records (sourced from GBIF and iNaturalist) indicated with green dots modelled with clamatic variables from South Africa. Warmer colours indicate high similarity between Europe and South Africa while cooler colours are less similar.

Climex models suggest that collecting insects when they have become accustomed to European climates may be a better match for South Africa than insects from the East coast of North America.

4.4. Discussion

Species distribution models or ecological niche models are excellent spatial-analysis tools to prioritize management areas for many different purposes (Santamarina *et al*., 2019). These include but are not limited to endangered species conservation, species distribution and compositional patterns, climate change predictions and the potential spread of biological invasions (Li *et al*., 2020; Mukherjee *et al*., 2011; Santamarina *et al*., 2019; Zhang *et al*., 2012). As prioritizing biological control agents is often referred to as the most time- consuming and expensive part of a biological control programme (Dhileepan *et al*., 2006; Fowler *et al*., 2000; Heard & Van Klinken, 2004), this study attempted to use models (MaxEnt and CLIMEX) to further prioritize the potential biological control agents identified in chapter 3 in order to save time and funds. These include *O. robiniae*, *M. robiniella* and *O. dorsalis*. This was done by modelling native range (North America) and invaded range (Europe) occurrence data combined for each potential biological control agent with environmental predictors in MaxEnt and projecting them geographically over South Africa using heat maps to visualise the results. By examining spatial patterns and infer potential ecological preferences of the species. Areas with high predicted suitability may represent core habitats, while transitions to lower suitability may indicate ecological barriers or unsuitable conditions. Therefore, heat maps, in ecological niche modeling, serve as a visual tool to communicate the model's predictions and provide insights into the potential distribution of a species based on environmental factors. These maps are valuable for conservation planning, habitat assessment, and understanding species-environment relationships.

Obolodiplosis robiniae and *M. robiniella* that have escaped their native range were modelled on the native range occurrences as well as their invaded rage occurrences as there is evidence that combining these data sets produces the best models (Trethowan *et al*., 2011). South African climatic data were also back projected over the native and invaded range where the occurrence data were sourced using CLIMEX modelling.

The projected model outcome for *O. dorsalis* had the highest potential suitability for establishment in South Africa, especially in the areas where the target weed, *R. pseudoacacia*, is most prevalent and damaging. There was some extrapolation but mostly interpolation in the area that was mostly the focus of this study, suggesting that these areas are likely to be favourable to the insect (Santamarina *et al*., 2019). *Macrosaccus robiniella* was second after *O. dorsalis* and showed some predicted suitability in South Africa, however, the model showed extrapolation over the same region that was predicted suitable for the insect. This is probably due to a combination of environmental conditions found in the suitable area that are not present in the native and invaded range where the occurrence records are from. This indicated the potential of inexact predictions and may be considered as less favourable for the insect. Lastly, *O. robiniae* was predicted as the least suitable biological control candidate based on the climate suitability models produced here to be potentially released in South Africa. Interpolation occurred in the predicted area and therefore there is less uncertainty compared to *M. robiniella,* resulting in a better prediction and suitability for the establishment of *O. robiniae* in South Africa. The CLIMEX models that have South Africa's climatic variables back projected over North America and Europe suggest that the climate in these areas where the occurrence records are found is very similar to South Africa's.

Both MaxEnt and CLIMEX are popular software programmes for creating ecological niche models. The difference between the two is that CLIMEX predicts a favourable climate of a selected area for the target species based on climate data, i.e., the observed distribution of the species determines the environment it's found in. MaxEnt uses climate data along with presence data (occurrence records) to determine the distribution potential of the target species based on maximum entropy distribution (Byeon *et al*., 2018; Early *et al*., 2022). In this study, the CLIMEX models seem to predict that the insects will have a higher likelihood of establishment due to similar climates between South Africa and North America and Europe compared to the results from the MaxEnt models. However, MaxEnt can determine the importance of each variable based on the occurrence data combined with the environmental predictors. CLIMEX only has the climate variables of South Africa projected over North America and

Europe which is the general area where the insects are found. For this reason, CLIMEX models may build in some bias such as, for example, multicollinearity. MaxEnt may therefore have an advantage as the presence data enables it to realize highly accurate classifications by analysing specific occurrence data from the target species to predict a potential distribution. The occurrence data are therefore a dependent variable as a function of environmental variables (Byeon *et al*., 2018).

It is important to note, however, that the results can be biased as model parameters are set by researchers and individual interpretation plays a role (Trethowan *et al*., 2011). Therefore models should be used with some caution while keeping model limitations and strengths in mind (Merow *et al*., 2013). One of the problems concerning ecological niche models is that the models are only as good as the occurrence data that has been collected to calibrate them with. When working with invasive species or potential biological control agents, obtaining native range data can be costly and difficult. Researches may accidently over sample or under sample certain areas, may face time and budget limitations or may be restricted by political or geographical boundaries. Biases may also be found in data collected via other sources depending on factors such as who collected the data, when it was collected and why it was collected (Trethowan *et al*., 2011).

From this study *O. robiniae* and *O. dorsalis* are recommended as the first insects to use as biological control agents for the management of *R. pseudoacacia* and should only be released in the eastern Free State until establishment can be confirmed. There are, however, some future studies that can be recommended from this chapter to increase accuracy and further identify the most suitable release locations for potential biological control agents in South Africa.

MaxEnt is able to apply other factors barring climate predictors such as land cover, soil, distance and other geographical variables (Byeon *et al*., 2018), and these may potentially influence the predictions made by the software algorithm. Therefore, modelling with more predictors and assessing the contribution of each one may corroborate or enhance these model results.

A retrospective study on these potential biological control agents may also result in valuable information on their physiological adaptation potential. *Obolodiplosis robiniae* escaped its native range and therefore was not moved to a novel environment on purpose (Duso *et al*., 2011; Kodoi *et al*., 2003; Weryielinger & Skuhrava, 2007). It was first observed in South Korea in 2002 (refer to chapter 3) and spread from there. Modelling its distribution over time and space from South Korea may indicate which variables, whether environmental or physiological, drove the species into new locations. Similarly, *M. robiniella* escaped its native range and was first observed in Italy in 1970 (Whitebread, 1990; Baugnée, 2014; Csóka *et al*., 2009). The factors that may have contributed to its spread throughout Europe are unknown and may indicate whether it will be able to establish in South Africa.

The "niche conservation hypothesis" suggests that species niches are conserved over time and space, and a study by Liu *et al*., (2020) largely confirmed this to be true. Their study provided evidence that little niche expansion occurred between native and introduced ranges and that the introduced species niche is similar to its native niche environmentally. These findings also support the use of ecological niche models over time and geographic space in response to climatic variables. Thus, in conclusion, *O. robiniae* and *O. dorsalis* are most likely to be initial targets for effective biological control agents as management for the invasive tree begins.

Based on this study, *O. robiniae* and *O. dorsalis* are recommended as potential biological control agents for managing *R. pseudoacacia*, with initial releases suggested in the eastern Free State. Further studies can enhance the accuracy of predictions by incorporating additional predictors and assessing their contributions. Retrospective studies on the physiological adaptation potential of these agents and investigating their spread in novel environments may provide valuable insights for future management strategies. Previous research supports the niche conservation hypothesis, indicating that introduced species generally maintain niches similar to their native ranges. Thus, ecological niche models can be effective tools for predicting species responses to climatic variables over different geographic locations and time periods. In conclusion, *O. robiniae* and *O. dorsalis* are recommended as initial targets for biological control agents in the management of *R. pseudoacacia*. However, further research and considerations are needed to enhance accuracy, identify suitable release locations, and understand the physiological adaptations and niche conservation potential of these agents, as well as their host range, before they are considered for release.

Chapter 5. Using native and introduced range data to determine host specificity of candidate agent *Obolodiplosis robiniae*

5.1. Introduction

Before potential biological control agents can be released, the insects' fundamental and ecological host ranges need to be determined to ensure the safety of native, economically important and ornamental plants (Lesieur *et al.*, 2020). This is usually achieved through experimental host specificity testing. This according to Lesieur *et al*. (2020) and Van Klinken & Raghu (2006) can be the most costly and time consuming aspect of biocontrol programmes due to the number of plant species that have to be tested.

Host specificity testing is guided by phylogenetic relatedness of the target plants. This method was first explained by Wapshere (1974) as the "centrifugal phylogenetic testing method". It involves exposing plants - that are most closely related to the target species - to the potential biological control agent, then progressively moving to more distantly related plants until the host range has been tested (Wapshere, 1974). Wapshere (1974) also suggests that economically important or cultivated plants be tested as well. These include cultivated plants that are related to the weed, cultivated plants with little information on their pests or herbivores, cultivated plants that have evolved away from their natural herbivores, cultivated plants known to be vulnerable to insects closely related to the potential biological control agents and any plant the potential biological control agent has been recorded on. There have been efforts to improve these methods. Incorporating knowledge on "plant phylogenetic relationships" and insect behaviour and evolution concerning host-choice, will ultimately improve outcomes from biological control programmes (Briese, 2005).

5.1.1. Taxonomically related species

To modernise the approach based on taxonomic relatedness it is important to incorporate new findings and investigate how insects select their hosts (Briese, 2003; Downey *et al.*, 2021). DNA sequencing and building phylogenetic trees have improved significantly from the late 1980s, early 1990s which have implications for biological control (Taylor & Dhileepan, 2019). Host selection is based upon a set of physiological and behavioural responses that the insects will have to chemical, visual and tactile cues provided by the plants it encounters (Briese, 2003). In the case of specialist insects, hosts are selected by means of ecological and phenological adaptations that allow the insect to complete its life-cycle on the plant. Examples of insect-host relationships from Mitter *et al*. (1991) and Futuyma (2000) confirm that specific insect species co-evolved with their plant can recognise cues from their host. Plant species that are closely related possess similar morphology and chemistry and therefore emit similar cues that may attract those insects, serving as additional hosts. The development of host plant lineages, particularly in terms of plant chemistry, is intricately connected to instances of insects being documented on different hosts, indicating shifts in the lineages of specialized insects (Briese, 2003). There are other factors also to consider when selecting closely related plants for host-specificity tests, such as plant distribution, biochemistry and plant morphology (Heard *et al*., 1997), but these criteria can lead to valuable species being overlooked. Briese (2003) suggests that using phylogenetics alone to select closely related plants would result in a shorter, more precise list of test species.

The cultivated economically important species Wapshere (1974) suggests be tested during host-specificity trials also need to be updated. The first category mentioned by Wapshere (1974) is “cultivated plants botanically related to the target weed”. Briese (2003) suggests that this makes sense from a risk assessment perspective, but if these plants are closely related to the target weed, they would already be on the list of species to be tested. The next category designated by Wapshere (1974) is “cultivated plants for which there is little or no entomological or mycological knowledge”. Again Briese (2003) points out that if these plants were closely related to the target weed, they would already be on the list of species to be tested. If these plants are not related to the target weed, it would imply that a fault has been made in the phylogenetic selection process. Briese (2003) also notes that there are hardly any cultivated plants with economic value that are poorly studied with unknown herbivores. The third category from Wapshere (1974) is “cultivated plants which have evolved apart, or which for geographic or climatic reasons have not been extensively exposed to the candidate agent”. Studies have shown that when related plants have evolved apart, they are less likely to serve as hosts for insect herbivores. This is because the plants have diverged in characteristics that provide host cues to the insects. When related plants evolve apart, they may diverge in these cues, making it more difficult for insects to recognize them as suitable hosts (Briese, 2003). This is due to characteristic selection that deviate over generations and therefore host-cues change for the insects, especially specialist insects (Olckers & Hulley, 1991; Briese, 2003). The fourth category from Wapshere (1974) is "cultivated plants known to be attacked by organisms closely related to the candidate agent". Briese (2003) suggests that this is an unnecessary criterion when selecting test plants. This is because insects closely related to the potential biological control agent, according to phylogenetic data, may belong to large genera. Insects within these large genera may contain several lineages where insects have evolved and made shifts to other plant families. The last category from Wapshere (1974) is "any plants on which the candidate agent has been previously recorded". By conducting field surveys in the native and invaded range, literature records can be tested. Polyphagous insects can then be eliminated as well as oligophagous insects with an unacceptably broad range of hosts by using the centrifugal-phylogenetic method (Briese, 2003).

5.1.2. Infield studies

In South Africa for example, *Dasinuera pilifera* Kolesik (Diptera: Cecidomyiidae) was released on *Acacia baileyana* F. Muell. and *A. decurrens* Willd. (Fabaceae) without host specificity testing (Adair, 2004). This was only allowed as it was clearly shown through historical releases of similar agents, phylogenetic studies of both the midge and the plant and native range studies that it posed no risk if released in South Africa (Kleinjan, 2015).

5.1.3. Potential biological control agent – *Obolodiplosis robiniae*

Testing potential biological control agents within quarantine settings can often prove challenging as these facilities do not replicate real-world conditions. Thus, the host range of an insect can be difficult to determine under artificial conditions and therefore some potential agents may unnecessarily be rejected (Briese, 2005; Sutton *et al*., 2021). Introduced or native range field studies offer a more natural and representative indication of an insect’s host range. These studies are usually conducted in the pest's native range or in areas where the pest has established. They are essential for assessing the effectiveness and safety of the biological control agent under natural conditions and can be used to verifying the results obtained from laboratory tests (Olckers & Hulley, 1995). Sometimes only infield host-specificity tests can be conducted, such as when candidate agents are very difficult to rear or target host plants cannot be grown in quarantine. There is, however, no set protocol for these types of studies (Sutton *et al*., 2021).

Obolodiplosis is a genus of gall midges (Cecidomyiidae). It is monotypic, being represented by a single species, *Obolodiplosis robiniae*, commonly known as the locust gall midge. It was first described in the USA (Pennsylvania) in the 19th century. It has a broad distribution in the USA, is regarded to follow the distribution of *R. pseudoacacia* in North America and is therefore abundant in eastern States and more occasional in the Western States of USA (Mally *et al*., 2021).

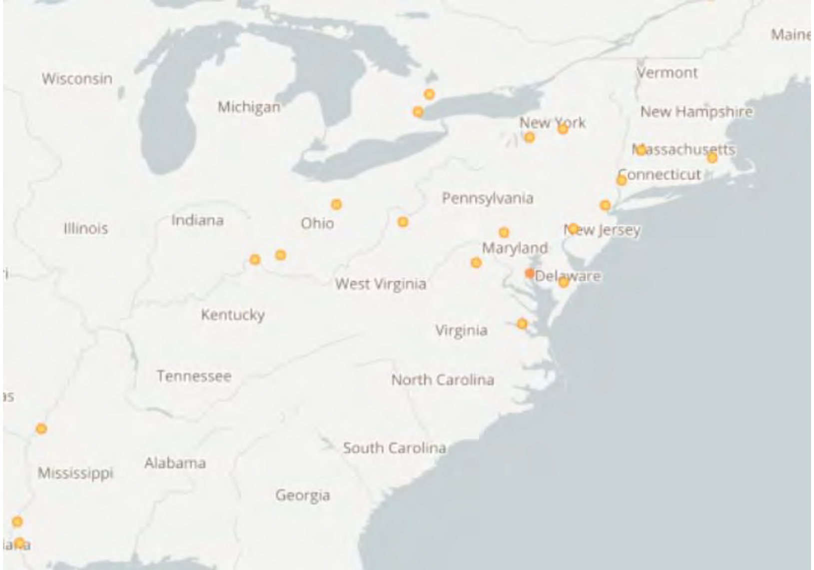


Figure 5.1. The distribution of *Obolodiplosis robiniae* in its native range, eastern North America. Species occurrence map from GBIF (https://www.gbif.org/)

Initially constrained to North America, the distribution of *O. robiniae* expanded upon its identification in Asia in 2002, notably within Japan and Korea, followed by its subsequent discovery in Europe's Italy in 2003. Since that time, it has disseminated across nearly all European nations where *R. pseudoacacia* is present, subsequently earning its status as an inadvertently introduced insect and consequently a pest, as indicated by Zhao *et al.* (2023). The midges have also been recorded in Russia, China and more recently New Zealand (Mally *et al.*, 2021). In the introduced range these midges can reach high densities (See Chapter 2: Bálint *et al.*, 2010; Mally *et al.*, 2021; Zhao *et al.*, 2022).

Figure 5.2. The years in which *Obolodiplosis robiniae* was discovered in various European countries (Bálint *et al.*, 2010)

Obolodiplosis robiniae poses a direct threat to the health of the tree due to larval feeding activity, which can result in damage to the tree. The leaf rolls, with larvae mining activity within, created by these insects can also weaken the tree's defences, making it more susceptible to secondary pest attacks. This cumulative damage can ultimately lead to the tree's demise (Zhao & Shin, 2019). For further information on *O. robiniae*, please refer to chapter 3 of this study.

This study aims to provide evidence based on extensive native and invaded range data as well as where the species has been introduced, that the potential biological control agent*, O. robiniae*, is sufficiently host specific to *R. pseudoacacia* to be considered a biological control agent in South Africa.

5.2. Materials and methods

To describe the host specificity of *O. robiniae,* evidence was derived from extensive research done on phylogenetic relationships within the sub family Papilionoideae and specifically the tribe Robinieae to which *R. pseudoacacia* belongs (for example: Hargrove, 1986; Kodoi *et al*., 2003; Zhao *et al*., 2022). Phylogenetic trees show the evolutionary relationships between different species based on their genetic similarities and differences. By comparing the genetic sequences of different species, we could identify which species are closely related to *R. pseudoacacia* and therefore may be more susceptible to attack by insects that feed on *R. pseudoacacia* in North America, Europe, and South Africa.

Studying the phylogenetic trees from Duan *et al*. (2020) and Su *et al*. (2020), the closest related plants to *R. pseudoacacia* were identified and those present in Europe, USA. These plants were found using GenBank (https://www.ncbi.nlm.nih.gov/) through the taxonomy filter, iNaturalist and GBIF (Global Biodiversity Information Facility) within the Robinioid clade. MEGA (Molecular Evolutionary Genetics Analysis) is a software programme commonly used to analyse and manipulate molecular data for phylogenetic analysis. One of the methods available in MEGA is the neighbour-joining algorithm, which is used to build phylogenetic trees based on genetic distance between sequences. Bootstrap is a statistical method used to estimate the reliability of a phylogenetic tree. It involves randomly sampling the original dataset multiple times to generate new datasets, and then reconstructing a new phylogenetic tree for each dataset. The resulting trees are then used to calculate the support for each branch of the original tree. The number of replications used in bootstrap analysis can vary, but 1000 is a commonly used number. The P-distance method is a measure of genetic distance that is calculated based on the proportion of nucleotide differences between two sequences. This method is often used for phylogenetic analysis of closely related organisms, such as within-species or within-genus comparisons. Overall, the process described uses MEGA software to align DNA sequences, construct a phylogenetic tree using the neighbour-joining algorithm, and assess the reliability of the tree using bootstrap analysis with 1000 replications and the P-distance method for genetic distance calculation.

Once we had identified species closely related to *R. pseudoacacia* in Europe and USA we could attempt to determine if any of those species were susceptible to attack from *O. robiniae*. To determine which species to further investigate we first had to narrow down the species list to species which shared a distribution with *R. pseudoacacia.* The occurrence data for the Robinoid clade and related species, including Robinia, were obtained from the Global Biodiversity Information Facility (GBIF) (Table 5.1, 5.2 and 5.3). To ensure data quality, a data cleaning process was conducted, which involved removing duplicate records, correcting coordinate errors when possible, excluding coordinates with insufficient precision, and excluding records before 1980 without subsequent updates in the corresponding areas. Refined occurrence locations for native and invaded range data of each species were visualized using ArcMap 10.8.1 for better understanding.

The refined occurrence data were then overlaid on the known distribution of *R. pseudoacacia* in Europe and the USA. Species with high occurrence correlations and at least 500 correlated records were given further consideration. Extensive literature searches were conducted using platforms such as Google, Google Scholar, Scopus, and EPPO to gather additional information about the refined species. Priority was given to species that had attributes enabling a better understanding of their associated insects or pests. Such attributes included being a crop species, commercially grown fodder species, a common ornamental species, having a biological control programme already considered for the species, or being a damaging weed species or an important forestry species.

In the results, specific symbols were used to represent each of the mentioned attributes of the investigated species. Please note that the symbols used in the results section represent the various attributes mentioned above.

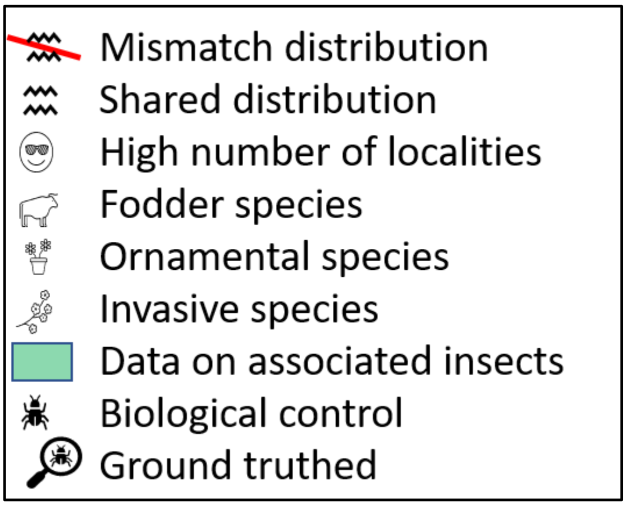


Figure 5.3. Symbols used to represent the correlated attributes for species further investigated.

Once species were refined extensive literature searches through Google, Google Scholar, Scopus and EPPO were used to identify whether herbivores that feed on *R. pseudoacacia*, could be found on any of the associated species. Keywords included species in question (e.g., *Sesbania sesban*), *Robinia pseudoacacia*/black locust, herbivores, associated insects, pests on the selected plants, insects found on the selected plants, agricultural pests, legume insects, etc. If substantial data on associated insects could be located that could support attack or non-attack by *O. robiniae* it was recoded.

5.2.1. In field host specificity testing

To confirm host specificity derived from the literature we conducted trials in both Europe and USA to investigate closely related species for non-target impacts caused by *Obolodiplosis robiniae*. In Europe, nine sites were selected in Switzerland and Germany with established *R. pseudoacacia* stands with *O. robiniae* infestations. Depending on the size of the *R. pseudoacacia* stand and site, quadrats (0.5 x 0.5m) were laid out and it was recorded how many quadrats were used at each site. Fabaceae species in the vicinity were then examined and the number of inspected species was recorded. The total amount of plants examined were also recorded as well as the number of *R. pseudoacacia* specialist herbivores found on other Fabaceae species. In USA approximately 500 *R. pseudoacacia* trees were sampled around the town of Blacksburg, Montgomery County, South West Virginia. At each site herbivory associated with *R. pseudoacacia* was recorded. In addition, surrounding vegetation was inspected for signs of similar damage. This was only conducted on dominant species surrounding *R. pseudoacacia* with high *O. robiniae* damage (Figure 5.4).

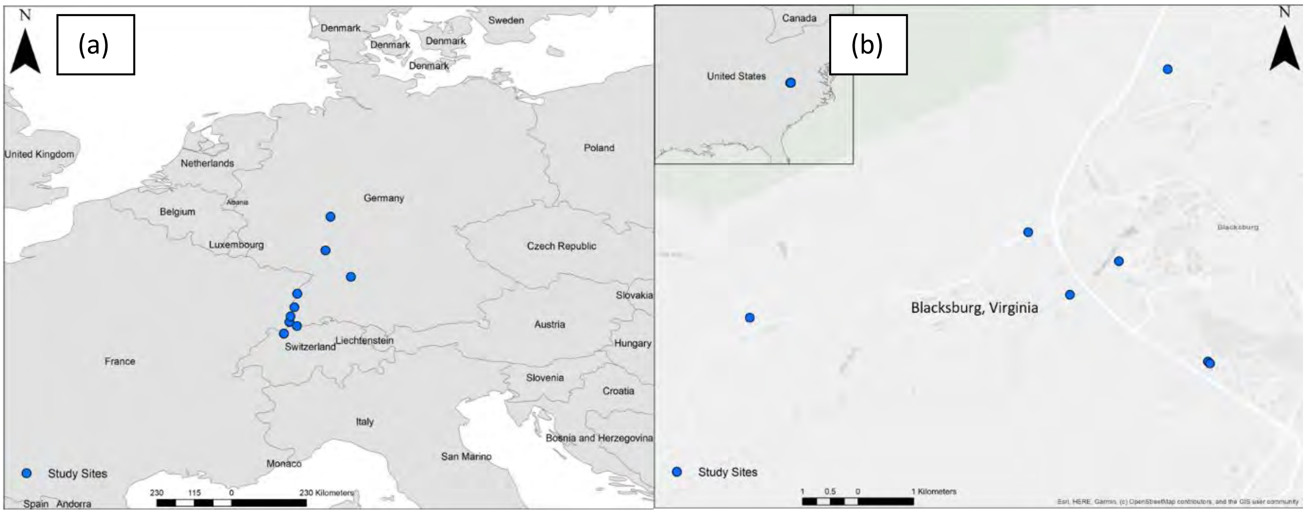


Figure 5.4. (a) Study sites where invaded range sampling of species closely related to *Robinia pseudoacacia* occurred in Europe. (b) Study sites where the same sampling took place in North America

5.2.2. Risk assessment of closely related species

As emphasized by Heard *et al*. (1997), it is prudent to contemplate the assortment of available plants in the targeted region when conducting a risk assessment for the potential introduction of a candidate agent. This comprehensive evaluation serves as a synthesis of the data discussed above. In line with this approach, plant species within South Africa were categorized into distinct groups according to their native or introduced status. Furthermore, their attributes, encompassing characteristics like trees, shrubs, and herbs, along with their geographical distribution, were taken into account. This strategic categorization aids in identifying which plants could be susceptible to unintended attacks by a specific species. To augment the assessment, factors influencing the likelihood of attack were examined, including morphological traits, shrub height, leaf size, and phylogenetic relationships. These attributes were employed to gauge the risk of feeding and whether instances of feeding had been documented on the plant species, either in Europe or the USA.

Test species consisted of the closest related species to *R. pseudoacacia* according to the phylogenetic tree as well as other common species within the Faboideae. Characteristics similar to *R. pseudoacacia* were given a point. At the end the fewer points a plant had the better were the chances that *O. robiniae* would not find it a suitable host. Characteristics examined include whether the plant is in the same genus or plant category (tree, shrub, herb).

5.3. Results

5.3.1. Phylogenetic relationships of Papilionoidea and *Robinia pseudoacacia*

The subclades within Papilionoideae are well supported in phylogenetic trees based on chloroplast genomes and *matK* gene sequencing, suggesting that these methods are effective for studying the evolutionary relationships within this subfamily (Cronk *et al*., 2006; Su *et al*., 2020). Within this subfamily, which is monophyletic, there are 10 clades. These include 50 kb inversion clade, Andira clade, dalbergioids, genistoids, NPAAA clade, Hologalegina, millettioid clade, mirbelioid clade, ADA clade and Cladrastis clade (Cronk *et al*., 2006). A partial phylogenetic tree of the Papilionoideae subfamily (Figure 5.5.) indicates the relationship of some of the major clades.

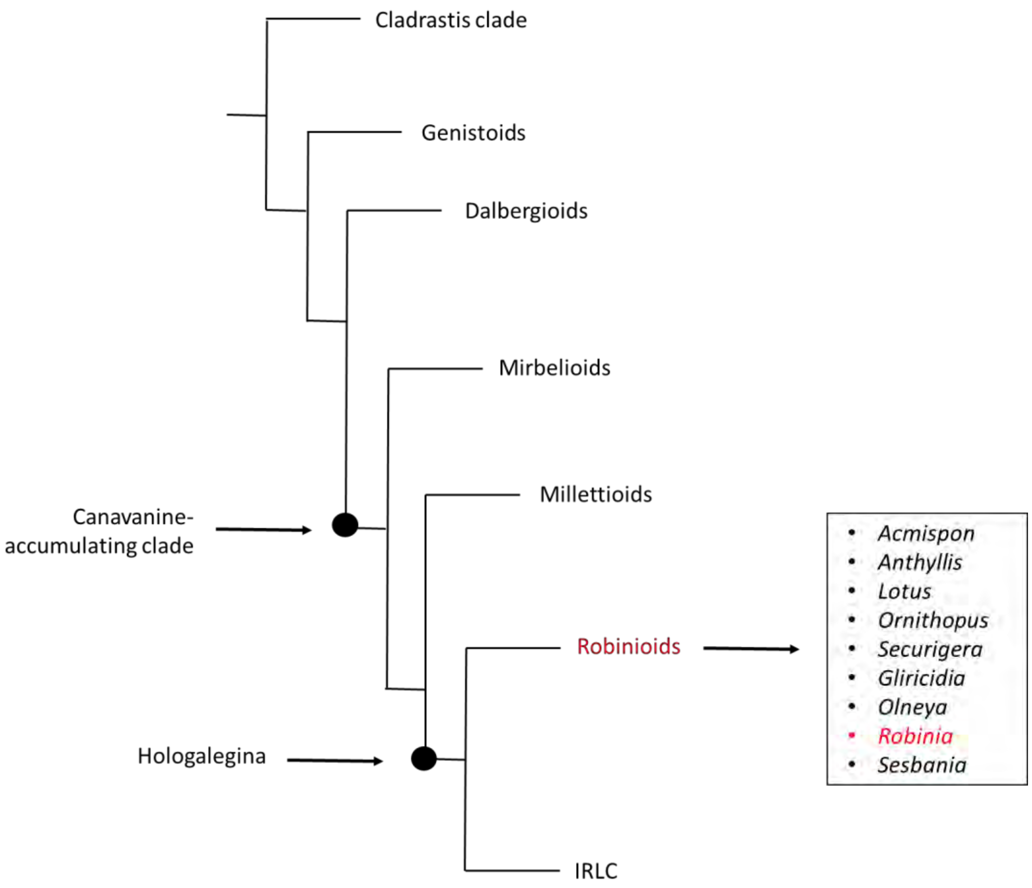


Figure 5.5. Partial tree (modified from Cronk *et al.*, 2006) representing clade relationships within the Papilionoideae Subfamily.

The Robinoid clade is represented in red as well as the Robinia genus that's also in red. Within this clade, the genus Robinia is found. The Square drawn from the Robinioid clade indicates the other genera present that are found in South Africa, Europe, and USA.

Within the *Robinia* Genus there are four *Robinia* species, namely *Robinia hispida* L., *Robinia neomexicana* A. Gray, *Robinia pseudoacacia* L. and *Robinia viscosa* Ventenat (Vinogradova *et al.*, 2013). There is also a variety, *Robinia neomexicana var. rusbyi* and an unclassified *Robinia* species, *Robinia sp*. SH-2010 (Cronk *et al.*, 2006). These species are listed in table 5.1. along with the generalised distribution for each genus. Except for *R. pseudoacacia*, no other *Robinia* species is found in South Africa.

Table 5.1. The generalised distribution of *Robinia* species globally.

Species	**Generalised distribution**
Robinia hispida	North America, South America*, Europe* and Asia*
Robinia neomexicana	North America, Europe* and Asia*
Robinia pseudoacacia	North America, South America*, Asia*, Africa*, Europe*, and Australia*
Robinia viscosa	North America, Europe* and Asia*

Spread outside of native range

Robinia pseudoacacia is classified under the Papilionoideae subfamily, which encompasses a vast diversity of plant species. Papilionoideae consists of 500 genera and over 14,000 species distributed across temperate, subtropical and tropical regions (Leeratiwong *et al*., 2020). In the southern African region, there are approximately 99 genera and 1,648 native species, 18 genera and 65 naturalized species, and an additional 61 genera and 264 cultivated species (Table 5.2). Within the Papilionoideae subfamily, *R. pseudoacacia* belongs to the robinioid clade, which includes the Loteae, Robinieae, and Sesbanieae clades (refer to Table 2.5.). In South Africa there are three recorded cultivated species each from the *Anthyllis* and *Hippocrepis* genera. Additionally, six species from the *Cornilla* genus are cultivated in the region. Under the *Lotus* genus there are four native species, two naturalized species, and eleven cultivated species in southern Africa. *Ornithopus sativus* is a naturalized species observed in the Western Cape, and two more species from the *Ornithopus* genus are cultivated in southern Africa (refer to Table 5.2). Furthermore, Southern Africa is home to several *Sesbania* species, with five species found in South Africa, including three naturalized species, and an additional two cultivated species (refer to Table 5.2). Please note that the specific information regarding genera, species counts, and their distributions in southern Africa can be found in Table 5.2.

Table 5.2. Species counts, distribution and additional information regarding species closely related to *Robinia pseudoacacia*

# of species Global	Genus	Species in South Africa	Notes
	Loteae		
24	Anthyllis	*barba-jovis*, tetraphylla*, vulneraria**	3 species cultivated
9	Coronilla	*cretica*, emerus*, glauca*, repanda*, vaginalis*, varia**	About 20 species (Atlantic islands, Mediterranean, Europe); six species cultivated in southern Africa
10	Hippocrepis	*comosa*, emerus*, unisiliquosa**	About 21 species (Europe, western Asia, Mediterranean); three species are cultivated in southern Africa.
147	Lotus	*discolor, mossamedensis, namulensism, wildii, corniculatus*, subbiflorus**	four species native to southern Africa, two species naturalised, and an additional 11 species that are cultivated in the region
6	Ornithopus	*sativus, pinnatus*, perpusillus **	About six species, native to Europe, Mediterranean region, western Asia, Atlantic Islands and South America. *Ornithopus sativus* is a naturalised species in the Western Cape, and there are an additional two species of *Ornithopus* that are cultivated in southern Africa.
	Robinieae		

4	Robinia	_Robinia pseudoacacia_	_Robinia pseudoacacia_ (Black locust, Witakasia) has become naturalised in southern Africa and is a declared Category 1B invasive plant in South Africa. In addition, _Robinia hispida_ (Rose acacia, Kelsey locust) is cultivated in USA.
	Sesbanieae		
52	Sesbania	_brevipedunculata, cinerascens, notialis, Sesban, tetraptera, transvaalensis, bispinosa_*, _punicea_*	Fifty-two species, native to warm regions and usually growing in wet places. Eighteen species native to southern Africa, three are naturalised and a further two are cultivated in the region. _Sesbania punicea_ (Red sesban) is a declared Category 1 invasive plant in South Africa

*** Indicates invasive species in South Africa**

5.3.2. Infield host specificity from Europe and USA

Phylogenetic trees of closely related species found in Europe (figure 5.6.) and USA (figure 5.7.) illustrate which plants to focus on for non-target attack from potential biological control agents for *R. pseudoacacia*. Note that *Lotus corniculatus* is not included due to lack of comparable genetic analysis.

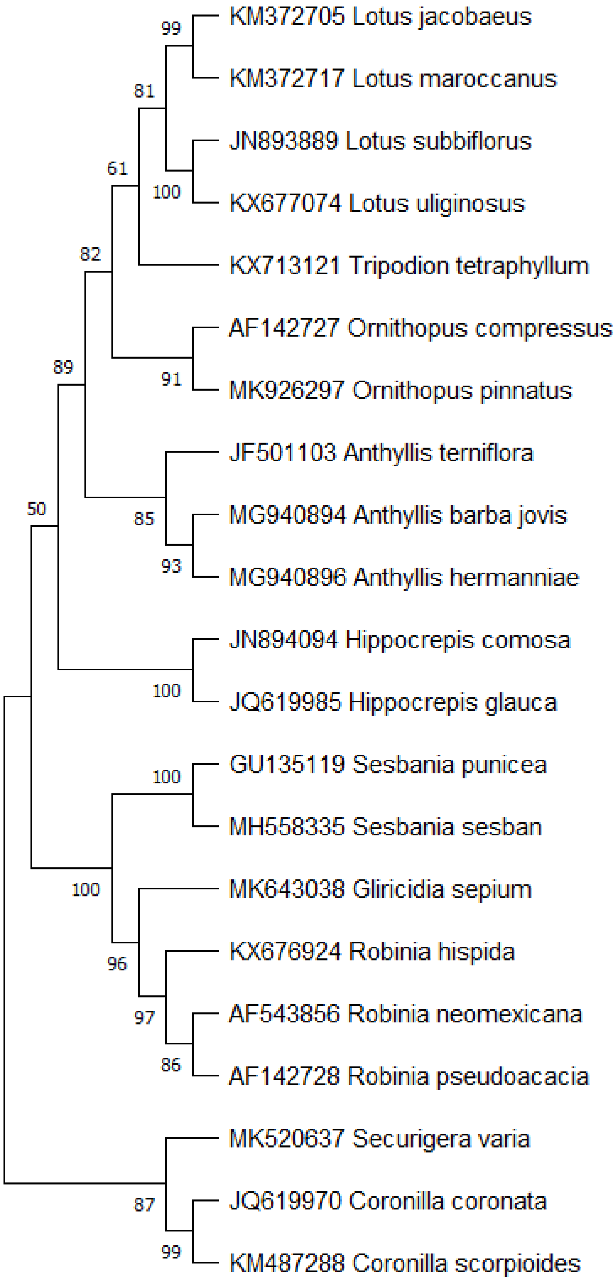


Figure 5.6. The phylogenetic tree of species within the Robinioid clade found in Europe based on *matK* chloroplast gene sequencing.

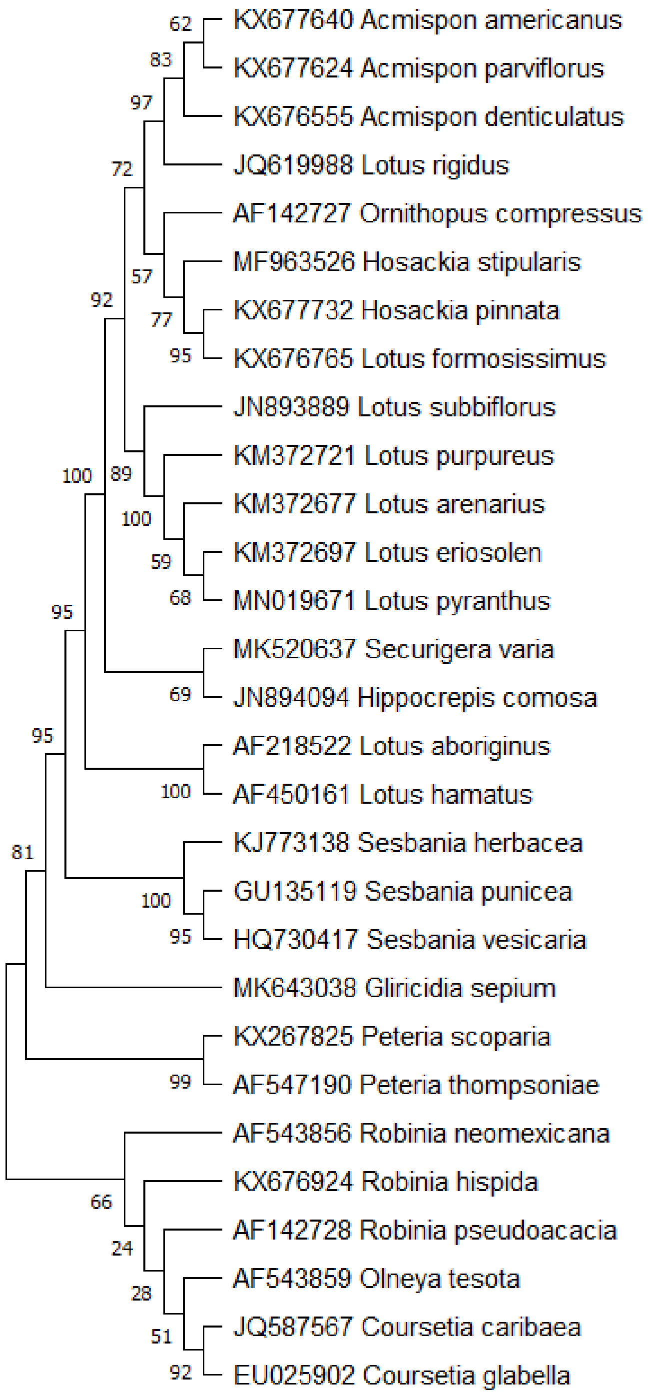


Figure 5.7. The phylogenetic tree of species within the Robinioid clade found in United States of America based on *matK* chloroplast gene sequencing.

5.3.3. Matching distributions

Of the 30 species in the Robinioid clade found in USA only 8 were found to share a significant distribution with *R. pseudoacacia*. Of those 8 species 4 were found to have over 500 shared occurrence records. The majority were found to be endemic to the West coast of the USA (figure 5.8). It is worth noting that some of the species are the same as those species found in South Africa.

Of the 20 species in the Robinioid clade found in Europe only 13 were found to share a significant distribution with *R. pseudoacacia* (figure 5.9). Of those 13 species 7 were found to have over 500 shared occurrence records. Again, it is worth noting that some of the species are the actual species found in South Africa.

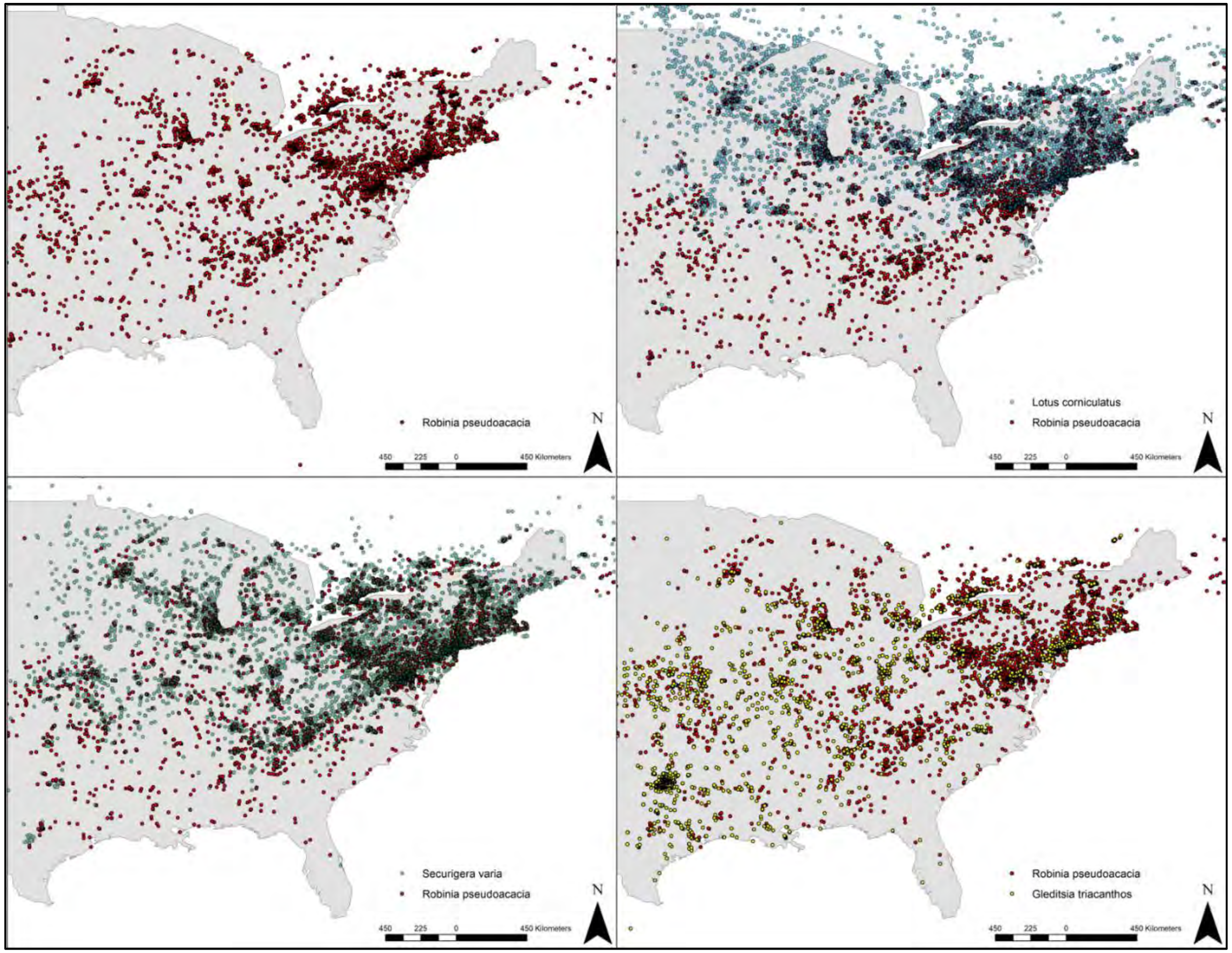


Figure 5.8. Distribution maps of the different plants closely related to *Robinia pseudoacacia* in North America generated by GBIF (https://www.gbif.org/) and visualised in ARC Map 10.3.

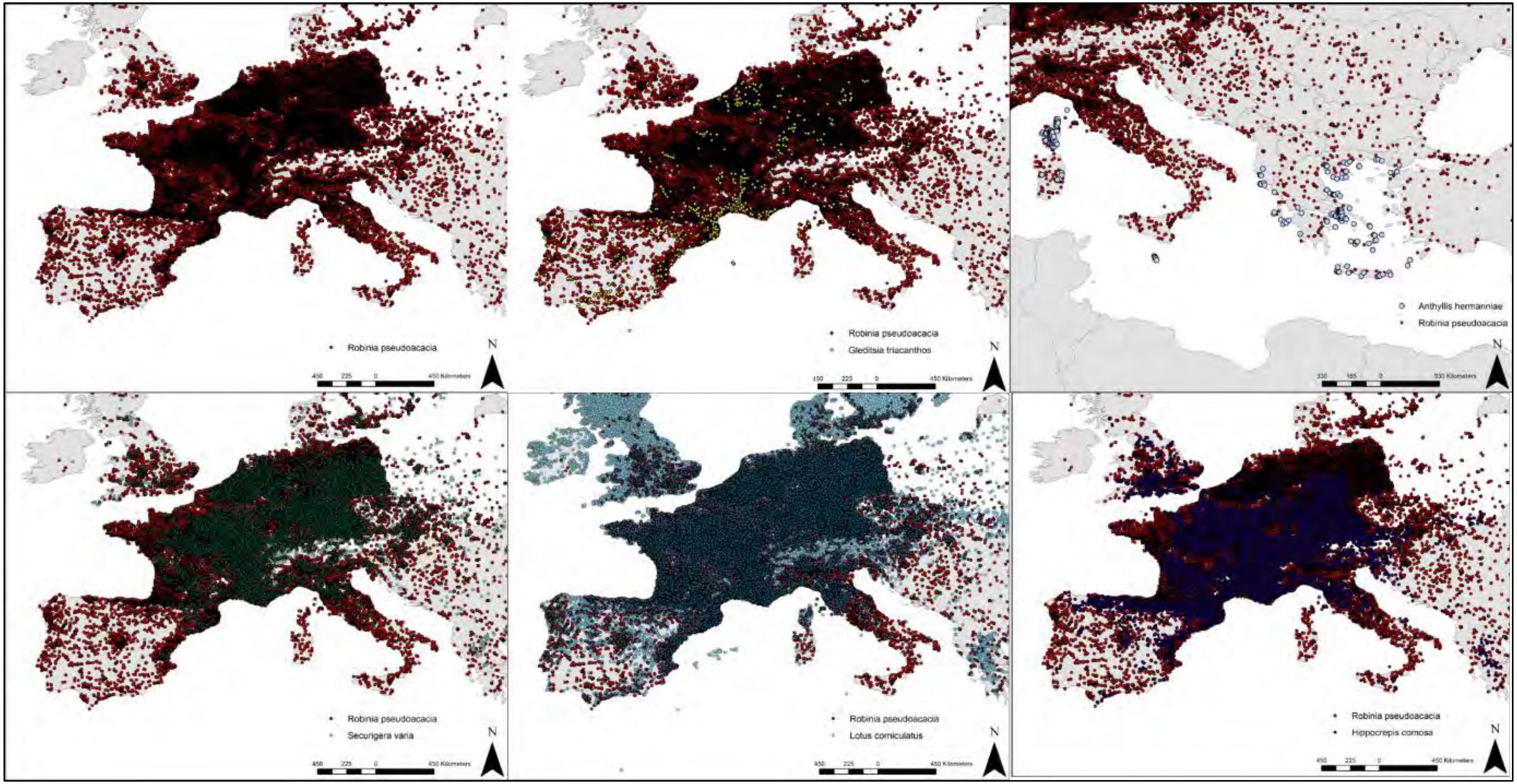


Figure 5.9. Distribution maps of the different plants closely related to *Robinia pseudoacacia* in Europe generated by GBIF (https://www.gbif.org/) and visualised in ARC Map 10.3.

Following the construction of the phylogenetic trees and reducing the number of species to those that just shared distributions and high numbers of occurrence records, the associated insects of those species were determined from extensive literature searches.

Securigera varia (L.) Lassen (Fabaceae: Papilionoideae) commonly known as crown vetch, is closely related to *R. pseudoacacia*. *Securigera varia* is native to Eurasia, and is a non-native species found throughout the contiguous United States and southern Canada. It is very common especially in the mid and eastern parts of USA (See figure 5.8) and has a strong distribution correlation with *R. pseudoacacia*. It is extensively used as a fodder species in the USA and occasionally for erosion control, but this is limited as the species tends to become invasive. Due to its importance, the species has been extensively studied. Wheeler (1980), for example, conducted surveys of the phytophagous arthropod fauna associated with *S. varia* in Pennsylvania, surveying large forage fields, highway plantings used for erosion control, and greenhouses. Emphasis was placed on determining what known pests of other forage legumes are associated with *S. varia* and on clarifying the relationship of the species to the host plant. Species were categorized as primary, secondary, and incidental according to their intimacy with the host. Biological notes, known host plant range, and association with other legumes were also taken. 125 phytophagous arthropods were collected in *S. varia* stands. None of these were *O. robiniae*. As it is a common agricultural species, associated pests are regularly reported to the Agricultural extensions in the USA (see https://extensionentomology.tamu.edu/resources/management-guides/managing-insect-pests-of-texas-forage-crops/vetch-insects). To date no record of *O. robiniae* has been made on the species. Finally, the species was considered in native range surveys. This species received the following categories for this study of note as it is not believed to be a host of the midge:

Figure 5.10. In this study, *Coronilla varia* received the following notable categories as it is not considered to be a host of the midge: *Obolodiplosis robiniae.*

Of the species that share distributions with *R. pseudoacacia* in USA (figure 5.11), sufficient data on associated insects could be collected for seven of the species. None of these species had any record of *O. robiniae*. A similar trend was found in Europe, where insect data could be located for 11 species and no record of *O. robiniae* was found (figure 5.11). Of the 46 species closely related to *R. pseudoacacia* in Europe and USA, data pertaining to the insects associated with the species could be located for 18 of 38 of the species. Of these insects, data showed 14 to be robust enough. This implies that *O. robiniae* was not associated with the species, while six were inconclusive. Data were unavailable for only 19 species and from 8 genera. These include: *Anthyllis barbajovis, Anthyllis terniflora Tripodion tetraphyllum, Lotus subbliflorus, Lotus macroccanus, Lotus hamatus, Lotus aboriginus, Lotus pyranthus, Lotus eriosolen, Lotus arenarius, Lotus purpureus, Lotus rigidus, Coursetia glabella, Peteria thompsoniae, Peteria scoparia, Hosackia pinnata, Hosackia stipularis, Acmispon denticulauts, Acmispon parviflorus* and *Acmispon americanus.*

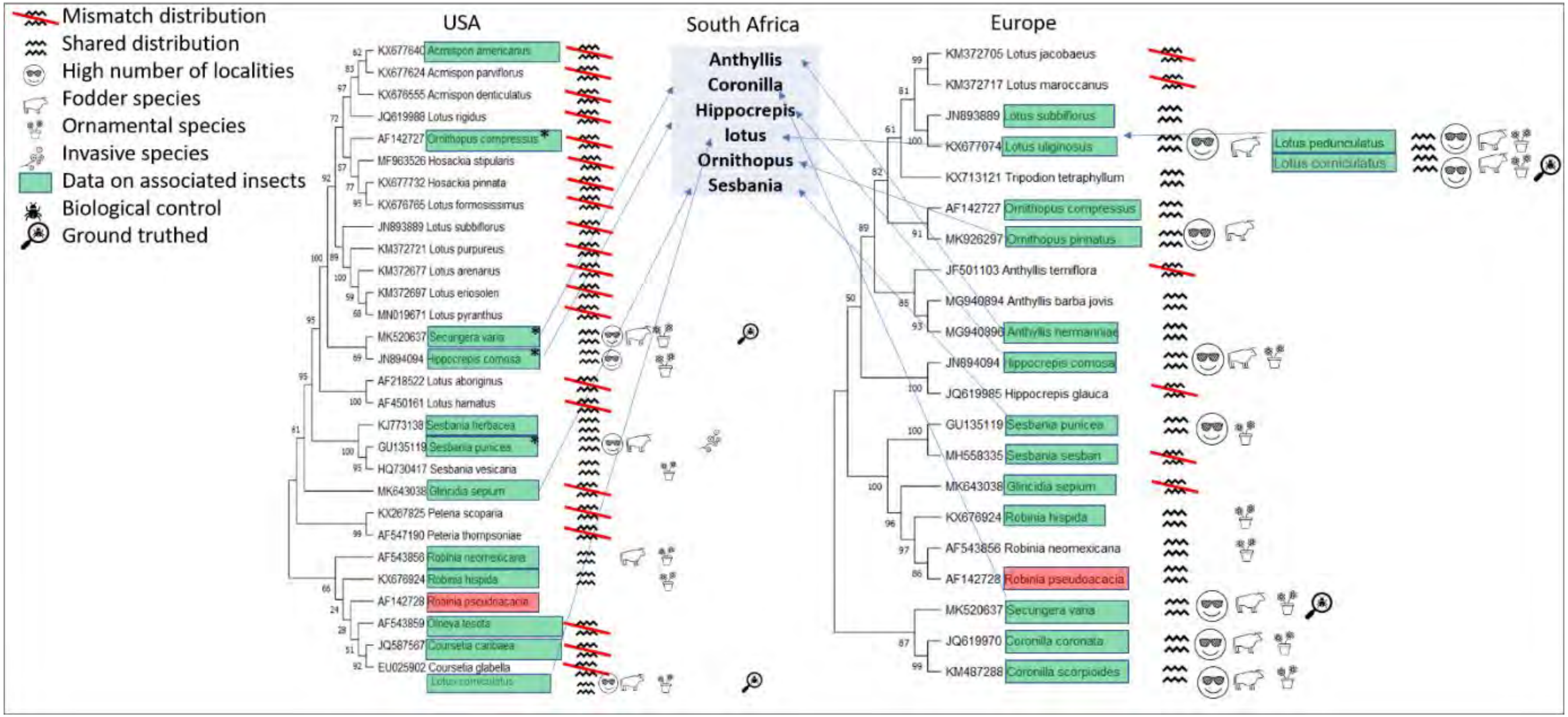
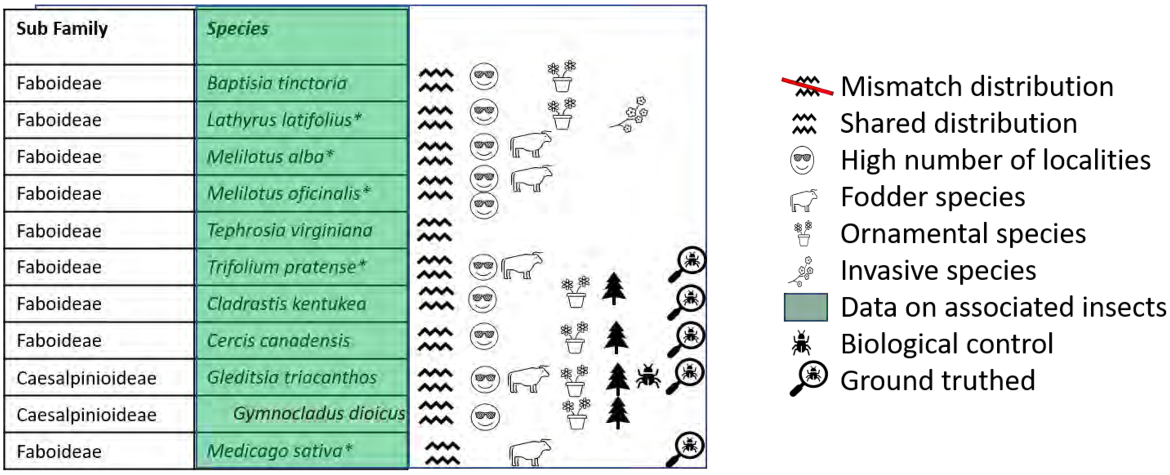


Figure 5.11. Phylogenetic trees depicting the interrelation among plant species closely akin to *Robinia pseudoacacia*, revealing connections based on specific criteria. These criteria encompass disparities in distribution, overlapping distributions, an extensive presence across multiple locations, designation as fodder or ornamental species, invasive tendencies, substantial data availability regarding associated insects, the feasibility of biological control, and corroborated empirical evidence.

5.3.4. Additional closely related species

In addition to the species within the Robinoid clade other abundant closely related species within the Faboideae and Caesalpinioideae were considered in the USA and Europe for associated insects. These species were selected primarily due to a certain attribute which would result in their associated insects being known. In the USA an additional 9 species within the Faboideae and 2 within the Caesalpinioideae were considered. Despite in some cases having significant associated literature of known insect pests no records of *O. robiniae* were located.

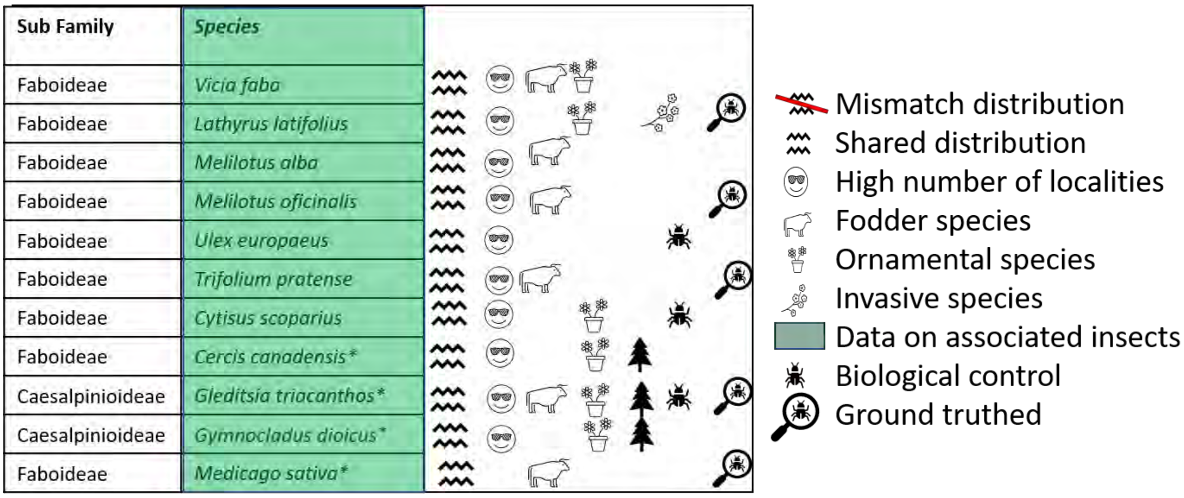


Sub Family	*Species*	
Faboideae	*Baptisia tinctoria*	〰 😎 🪴
Faboideae	*Lathyrus latifolius**	〰 😎 🪴 🌸
Faboideae	*Melilotus alba**	〰 😎 🐄
Faboideae	*Melilotus oficinalis**	〰 😎 😎 🐄
Faboideae	*Tephrosia virginiana*	〰
Faboideae	*Trifolium pratense**	〰 😎 🐄 🔍
Faboideae	*Cladrastis kentukea*	〰 😎 🪴 🌲 🔍
Faboideae	*Cercis canadensis*	〰 😎 🪴 🌲 🔍
Caesalpinioideae	*Gleditsia triacanthos*	〰 😎 🐄 🪴 🌲 🐞 🔍
Caesalpinioideae	*Gymnocladus dioicus*	〰 😎 🪴 🌲
Faboideae	*Medicago sativa**	〰 🐄 🔍

~~〰~~ Mismatch distribution
〰 Shared distribution
😎 High number of localities
🐄 Fodder species
🪴 Ornamental species
🌸 Invasive species
🟩 Data on associated insects
🐞 Biological control
🔍 Ground truthed

Figure 5.12. Plants (Sub Family and species) closely related to *Robinia pseudoacacia* in USA that comply with certain criteria in order to establish whether or not they could be targeted as potential hosts by *Obolodiplosis robiniae*.

In Europe an additional 9 species within the Faboideae and 2 within the Caesalpinioideae were also considered. Again, no record of *O. robiniae* was recorded.



Sub Family	Species
Faboideae	*Vicia faba*
Faboideae	*Lathyrus latifolius*
Faboideae	*Melilotus alba*
Faboideae	*Melilotus oficinalis*
Faboideae	*Ulex europaeus*
Faboideae	*Trifolium pratense*
Faboideae	*Cytisus scoparius*
Faboideae	*Cercis canadensis**
Caesalpinioideae	*Gleditsia triacanthos**
Caesalpinioideae	*Gymnocladus dioicus**
Faboideae	*Medicago sativa**

Figure 5.13. Plants (Sub Family and species) closely related to *Robinia pseudoacacia* in Europe that observe certain criteria in order to establish whether or not they could be targeted as potential hosts by *Obolodiplosis robiniae.*

In addition to the closely related species, sufficient data were found that closely related economically important species (that share distributions in both Europe and USA) are not hosts for *O. robiniae*.

5.3.5. Native range surveys

Surveys in USA considered species growing close to sites where *O. robiniae* was being sampled and at times considered distantly related species. However, *R. pseudoacacia* was not recorded. Data from native range surveys are also recorded on the figure (Figure 5.13.).

Table 5.3. The number of sites sampled in Switzerland with the number of galls containing *O. robiniae* found on plants closely related to *R. pseudoacacia*.

Family	*Plant Species*	Number of sites	Number of galls containing *O. robiniae*
Faboideae	*Lotus corniculatus*	2	0
Faboideae	*Securigera varia*	4	0
Faboideae	*Medicago sativa*	2	0
Faboideae	*Trifolium sp*	4	0
Faboideae	*Trifolium repens*	4	0
Faboideae	*Cladrastis kentukea*	3	0
Faboideae	*Cercis canadensis*	3	0
Caprifoliaceae	*Lonicera spp.*	2	0
Poaceae	*Phalaris arundinacea*	2	0
Elaeagnaceae	*Elaeagnus spp.*	2	0
Caesalpinioideae	*Gleditsia triacanthos*	6	0
Phytolaccaceae	*Phytolacca americana*	2	0
Caprifoliaceae	*Dipsacus fullonum*	2	0
Apiaceae	*Cicuta maculata*	2	0

Data from Switzerland (table 5.3) indicate the occurrence data of *O. robiniae* related with *R. pseudoacacia* found on other Fabaceae plants in the vicinity of *R. pseudoacacia* stands with *O. robiniae* infestations. Followed by table 5.4 that indicates the number and species of Fabaceae plants that were examined. Additional information can be found in appendix 5.3.

5.3.6. Likelihood of specificity in South Africa

To identify which plants are at risk for non-target attack, a phylogenetic tree (figure 5.14) was constructed using plants closely related to *Robinia pseudoacacia* found in South Africa.

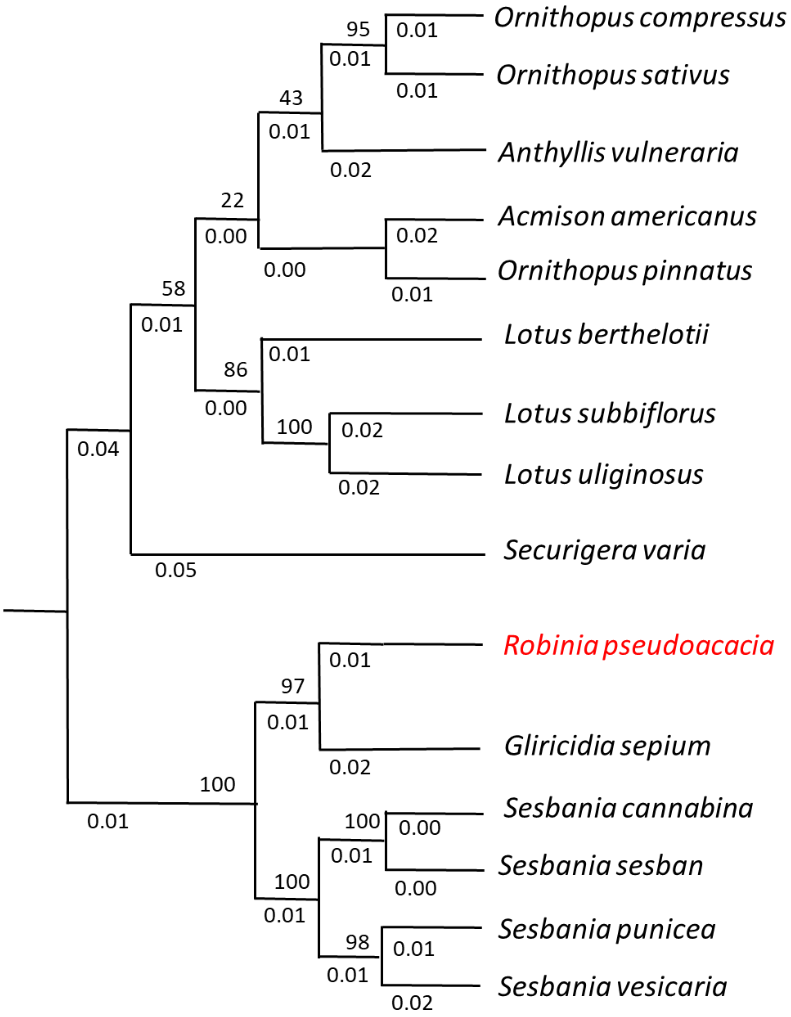


Figure 5.14. The phylogenetic tree of species within the Robinioid clade found in South Africa based on *matK* chloroplast gene sequencing. (All plants in the above phylogenetic tree are not native to South Africa. See table 5.4. for native plants.)

From this phylogenetic tree, table 5.4. was drawn to indicate whether these closely related plants are introduced, their morphology and their distribution.

Gliricidia sepium (fence post tree) is the closest relative to *R. pseudoacacia* that shares similar morphology in South Africa. However, it is considered an invasive tree and requires management. Only two other native plants, *Sesbania transvaalensis* and *Lotus discolour,* that are closely related to *R. pseudoacacia* are native to South Africa. Table 5.5 compares their leaf morphology with that of *R. pseudoacacia*. There are no suitable hosts for *O. robiniae* from this list.

Table 5.4. Plants closely related to *Robinia pseudoacacia*, whether indigenous or introduced, along with their morphological traits and geographical distribution in South Africa.

Plant species	**Common name**	**Native**	**Introduced**	**Tree**	**Shrub**	**Herb**	**Overlapping distribution in SA**
Gliricidia sepium	Fence Post Tree		x	x			No
Sesbania bispinosa	Prickly Sesban		x		x		No
Sesbania cannabina	Sesbania Pea		x		x		No
Sesbania punicea	Scarlet Sesban		x		x		Yes
Sesbania sesban	Egyptian Riverhemp		x		x		No
Sesbania transvaalensis		x			x		Yes
Securigera varia	Purple Crownvetch		x			x	Yes
Lotus corniculatus	Bird’s-foot Trefoil		x			x	Yes
Lotus discolor	Coral Plant	x				x	Yes
Lotus pedunculatus	Greater Bird’s-Foot-Trefoil		x			x	Yes

Lotus subbiflorus	Hairy Bird’s-Foot-Trefoil	x	x	Yes
Ornithopus compressus	Yellow Serradella	x	x	No
Ornithopus pinnatus	Orange Bird’s-Foot	x	x	No
Ornithopus sativus	Serradella	x	x	Yes

Table 5.5. The comparison of *Robinia pseudoacacia* to closely related plants that are native to South Africa. Leaf length is compared as a means to establish whether a biological control agent would be able to survive on these plants based on morphological similarity.

Plant species	**Common name**	**Native**	**Tree, shrub, herb**	**Leaf length**	**Morphologically suitable**
Robinia pseudoacacia	Black locust		tree	25 – 50 mm	-
Sesbania transvaalensis	-	x	shrub	1.5 – 3 mm	No
Lotus discolor	Coral Plant	x	herb	10 – 15 mm	No

To illustrate the morphological difference in leaf sizes between herbs and the trees, the following images were compared between the *Lotus* species (iNaturalist) and *R. pseudoacacia* (Figure 5.15).

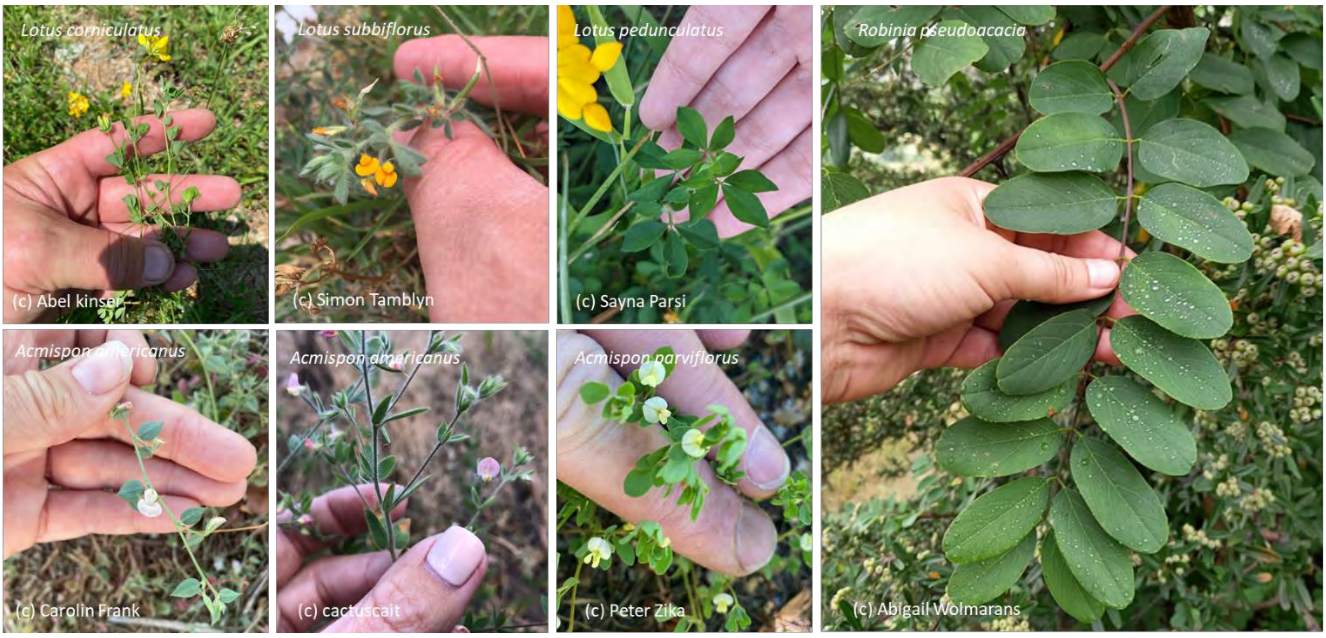
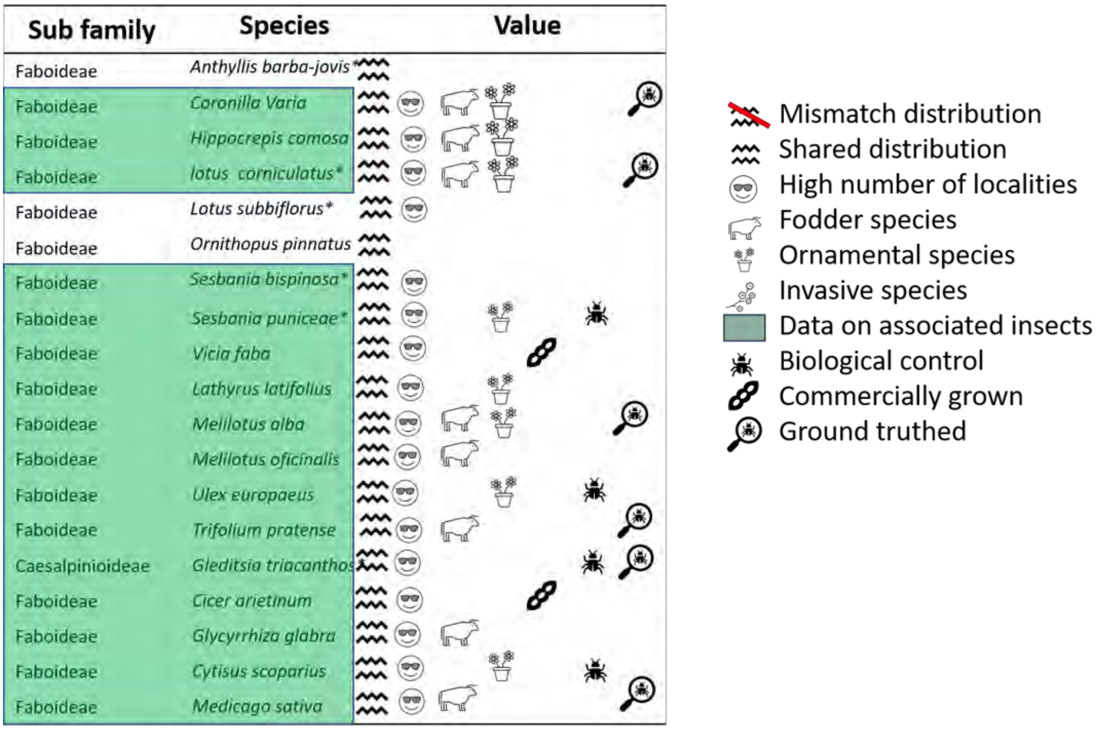


Figure 5.15. The six species on the left are herbs from the *Lotus* and *Acmispon* genera (iNaturalist) compared to *Robinia pseudoacacia* on the right. They will most probably not be able to support the growth of *Obolodiplosis robiniae,* that needs to roll the leaves in order for the larvae to grow due to leaves being too small.

In South Africa (Figure 5.16), a total of 18 species within the Faboideae Clade and 1 species within the Caesalpinioideae were investigated. Within none of the clades or families were any suitable host plants found for *O. robiniae*. Thus, no species in South Africa will be threatened by *O. robiniae*, as the exact species has been surveyed in the USA or Europe.



Sub family	Species	Value
Faboideae	*Anthyllis barba-jovis**	≈
Faboideae	*Coronilla Varia*	≈ 😎 🐄 ❀ 🔍
Faboideae	*Hippocrepis comosa*	≈ 😎 🐄 ❀
Faboideae	*lotus corniculatus**	≈ 😎 🐄 ❀ 🔍
Faboideae	*Lotus subbiflorus**	≈ 😎
Faboideae	*Ornithopus pinnatus*	≈
Faboideae	*Sesbania bispinosa**	≈ 😎
Faboideae	*Sesbania puniceae**	≈ 😎 ❀ 🪲
Faboideae	*Vicia faba*	≈ 😎 🔗
Faboideae	*Lathyrus latifolius*	≈ 😎 ❀
Faboideae	*Melilotus alba*	≈ 😎 🐄 ❀ 🔍
Faboideae	*Melilotus oficinalis*	≈ 😎 🐄
Faboideae	*Ulex europaeus*	≈ 😎 ❀ 🪲
Faboideae	*Trifolium pratense*	≈ 😎 🐄 🔍
Caesalpinioideae	*Gleditsia triacanthos*	≈ 😎 🪲 🔍
Faboideae	*Cicer arietinum*	≈ 😎 🔗
Faboideae	*Glycyrrhiza glabra*	≈ 😎 🐄
Faboideae	*Cytisus scoparius*	≈ 😎 ❀ 🪲
Faboideae	*Medicago sativa*	≈ 😎 🐄 🔍

≉ Mismatch distribution
≈ Shared distribution
😎 High number of localities
🐄 Fodder species
❀ Ornamental species
🌿 Invasive species
🟩 Data on associated insects
🪲 Biological control
🔗 Commercially grown
🔍 Ground truthed

Figure 5.16. Plants (Sub Family and species) closely related to *Robinia pseudoacacia* in South Africa that fulfil certain criteria in order to establish whether or not they could be targeted as potential hosts by *Obolodiplosis robiniae*

5.4. Discussion and conclusion

This study focuses on determining the host specificity of *O. robiniae*, a gall midge, particularly in its introduction to South Africa. The challenges and costs associated with experimental host specificity testing are highlighted in the introduction, with an emphasis on the guidance provided by phylogenetic relatedness. The taxonomic approach is proposed for modernization, incorporating advancements in DNA sequencing and considering physiological and behavioural responses of insects to host plants. The study critically evaluates the criteria for selecting test plants and advocates for infield studies in native and invaded ranges to provide a more realistic assessment of an insect's host range. Through an extensive examination of *O. robiniae's* distribution, introduction history, and impact, the aim is to offer evidence supporting its host specificity to *R. pseudoacacia* in South Africa in order to potentially release a biological control agent without conducting host specificity test.

Biological control has been successful for several years (Barratt *et al.*, 2018), but it remains important to evaluate the potentially harmful effects of introduced insects for biological control (Simberloff & Stiling, 1998). Gall-forming organisms, including those within the Cecidomyiidae family such as *O. robiniae,* are mostly specialised to a single host or closely related hosts (Shorthouse *et al.*, 2005; Carneiro *et al.*, 2009; Hardy & Cook, 2010; Miller & Raman, 2019; Park *et al.*, 2019; Santos-Silva & Araújo, 2020) making them attractive biological control prospects. From the World Catalogue of Agents and Their Targets there are 19 biological control agents belonging to the Cecidomyiidae Family (Winston *et al.*, 2014). *Obolodiplosis robiniae* follows suit as it supports the notion to be host-specific to *R. pseudoacacia* (Ilea *et al.*, 2021).

This chapter is based on morphological and phylogenetic evaluation of *R. pseudoacacia* and one of its specialised herbivores, *O. robiniae*. *Robinia pseudoacacia* is an aggressive transformer of the grassland ecosystem in South Africa and since it spreads by root suckering and less so by seed dispersal, the leaf-galling behaviour of *O. robiniae* is ideally suited to inhibit the spread and health of its host. The fact that there are no species from the *Robinia* genus in South Africa provides evidence that releasing a biological control agent specific to that genus in South Africa would be safe. *Obolodiplosis robiniae* is associated exclusively with the leaves of *R. pseudoacacia* and poses no threat to native or commercial flora in South Africa. The evidence is derived from literature reviews and experimental studies in its introduced range. The source confidence is also positive and can be relied upon.

Obolodiplosis robiniae has become established and documented in several countries and continents and is likely to spread rapidly as it did in Europe (Mally *et al.*, 2021). According to the study from Mally *et al.* (2021), the success of *O. robiniae* in the introduced range is attributed to its capacity to make long-distance dispersal jumps, high reproduction rates and limited parasitoid species and can spread up to 128 km/year. However, according to Tóth *et al.*, (2009) those parasitoids associated with the midge, although not many species, cause substantial damage to the population and therefore the midge does not have a great effect on *R. pseudoacacia* stands in Europe. The growth and reproduction rate of *R. pseudoacacia* may be higher than the rate at which the midge can cause damage as well.

Obolodiplosis robiniae is strictly a leaf-feeding agent (Mally *et al*., 2021) and does not affect the wood of *R. pseudoacacia*, the only guild of the tree that is used to some degree (Chikowore *et al*., 2021a). *Robinia pseudoacacia* is also not planted commercially in South Africa and from a study done by Chikowore *et al*. (2021a) it was found that rural settlements, where the tree is most abundant, have not integrated *R. pseudoacacia* into their livelihoods and only use the wood as a last resort. Management through biological control is therefore unlikely to disadvantage these communities or commercial stakeholders (Chikowore *et al*., 2021a).

Robinia pseudoacacia has invaded most temperate climate regions of the world, conditions that are beneficial to the establishment of its herbivores. This, combined with the fact that cargo traffic has increased during the last decade increases the likelihood for non-native herbivores to spread over large distances (Mally *et al*., 2021), *O robiniae* likely to be one of them in the future.

This evidence that *O. robiniae* is highly specialised to *R. pseudoacacia* and that the closest genetic relative to *R. pseudoacacia* in South Africa is too far along the phylogenetic tree to sustain *O. robiniae* supports the hypothesis that *O. robiniae* will be safe for release without traditional quarantine host-specificity tests. Other plants within the same clade, subfamily, and family as *R. pseudoacacia* found in USA, and Europe, such as the *Lotus* and *Sesbania* species that are classified as herbs and shrubs show no sign of attack from the midge, even where the midge is found in abundance.

From studies on herbivores documented on close relatives of *R. pseudoacacia* in its native range (USA) and areas where both the tree and midge are found (EU), it has been found that *O. robiniae* has not been documented on any of these plants (especially in areas where there is overlap of the plants). Further investigation to identify morphological similarities between *R. pseudoacacia* and its five closest relatives found that it is highly unlikely that the midge would find any of these plants a suitable host. This strengthens the argument that it would be safe to release *O. robiniae* without traditional host specificity tests.

The closest relatives to *R. pseudoacacia* in South Africa are herbs and shrubs. Since *O. robiniae* has co-evolved with *R. pseudoacacia* to be able to roll its leaves it would be unlikely that the midge would be able to do so with another plant as they are highly specialized towards their hosts (Shorthouse *et al*., 2005). Thus, it is predicted that plants belonging to the herb and shrub category would be safe in the event of *O. robiniae* being released as a biological control agent. Since shrubs are larger than the ground cover herbs, and there is only one native shrub closely related to *R. pseudoacacia* in South Africa, the data show that *O. robiniae* is safe to release as a biological control agent. However, it would still be possible to test *Sesbania transvaalensis* as a safety measure if need be.

Chapter 6. General Discussion and Conclusion

6.1. Introduction

Developing biological control agents for the management of invasive plants is no easy task. The most important component of a control programme is arguably determining the host range of selected and imported agents. Predicting the impact of potential agents is probably the most underdeveloped part of biological control (Goolsby *et al*., 2004). This thesis, through an ecologically motivated, multi-faceted approach, aimed to underline the importance of prioritizing biological control agents prior to importation while focusing on the invasive tree *Robinia pseudoacacia* that currently has no native herbivores present on it in South Africa.

The study aimed to address research questions and objectives related to the management of the invasive tree *R. pseudoacacia* in South Africa. These inquiries defined understanding the host range of potential biological control agents, examining conflicts of interest among stakeholders, assessing the utilization and impact of *R. pseudoacacia* on local communities, identifying associated insects and reproduction modes, prioritizing biological control agents through climate-matching models, and evaluating the safety of releasing *O. robiniae* without traditional host-specificity tests. The study also sought to contribute insights into *R. pseudoacacia* in the Southern Hemisphere and explore approaches, such as integrating citizen science, literature reviews, and predictive modeling, for prioritizing biological control agents. The overarching objective was to develop strategies for managing *R. pseudoacacia* while considering ecological, economic, and social factors in the South African context.

6.2. Discussions with appropriate interested and affected parties

Plants have been introduced from their native ranges to other countries for specific uses but often over time, these plants are used for other purposes as well (Chikowore *et al*., 2021b). For example, *Acacia cyclops* (Fabaceae) introduced for dune stabilisation is now used domestically as well as commercially as fuelwood (Chikowore *et al*., 2021b). Not all alien species have been introduced with a purpose, some species have spread accidentally such as *Bidens pilosa* L. (Asteraceae), which is a common agricultural weed but is used for food and medicine (Bartolome *et al*., 2013; Chikowore *et al*., 2021b). Often communities start using alien species as a result of the decline of native species, and this is not due to these species having more sought- after traits (Geesing *et al*., 2004; Shackleton *et al*., 2007a; Shackleton *et al*., 2007b). Conflict between different stakeholders can arise between negative environmental impacts of the weed and community use and needs and therefore context is important when these impacts and benefits are weighed (Shackleton *et al*., 2019). Plantation trees for example benefit private and national economies, but have a negative impact when they escape onto community land and therefore trade-offs between benefits and negative impacts need to be made (Ewel *et al*., 1999; Chikowore *et al*., 2021b). For example, in South Africa, *Acacia mearnsii* (Fabaceae) has more negative impacts on the environment than economic benefits (Chikowore *et al*., 2021b). Evaluations in South Africa have reported both positive and negative impacts from invasive alien trees on rural communities (Chikowore *et al*., 2021b). With regard to biological control, a significant conflict of interest may sometimes completely stop a biological control programme and prevent the release of biological control agents (see Hoffman *et al*., 2011 for example).

"Beloved yet despised" is how *R. pseudoacacia* is often described, primarily in Europe (Vítková *et al*., 2017). This is due to its conflicting nature as it has an economic value such as erosion control, rot-resistant timber and honey production but is also known to be very invasive due to its negative impacts on biodiversity, rapid spread and how difficult it is to control (Cierjacks *et al*., 2013; Chikowore *et al*., 2021). It is therefore expected that there might be conflicts of interest associated with its management in South Africa and thus conflicts should be considered before starting any biological control programme. *Robinia pseudoacacia* has spread to all nine provinces in South Africa but according to Martin (2019), it has invaded and impacted the grassland biome the worst. Rural inhabitants of the grasslands in South Africa make use of ecosystem services as means of support in their communities, for example, the use of thatching grass for construction materials and sweeping brooms as well as other plants in the ecosystem that are used for medicines (Wisely *et al*., 2018; Chikowore *et al*., 2021b) . However, in undisturbed grasslands trees are scarce and provide services that these communities also require such as construction materials, fencing posts and firewood, and are therefore capitalised on when available whether they've been planted intentionally or not (Chikowore *et al*., 2021b). This is evident in the montane grasslands of South Africa where many alien trees are found to satisfy societal needs (Chikowore *et al*., 2021a). These trees are often invasive and it is argued that they have a competitive advantage as they have no natural enemies (Chikowore *et al*., 2021a). Conflict often arises around the control of these species that not only include *R. pseudoacacia* but also *Acacia mearnsii* (Fabaceae), *Pinus pinaster* (Pinaceae), *Eucalyptus globulus* (Myrtaceae) and *Acacia saligna* (Fabaceae) for example (Chikowore *et al*., 2021a). Although *R. pseudoacacia* has presented benefits to the communities, the negative impacts outweigh these as it may reduce gross market in livestock performances and reduces native vegetation biodiversity (Humphrey *et al*., 2019; Chikowore *et al*., 2021a).

Until now the potential contribution to livelihoods, despite its negative impacts, and the utilisation of *R. pseudoacacia* in South Africa has been undocumented. A recent study showed that rural communities in the grassland biome of South Africa make use of *R. pseudoacacia* to some extent, although it was considered opportunistic and *R. pseudoacacia* was not considered to be of high importance (Chikowore *et al*., 2021b). Since the trees make dense stands, the use was driven by availability instead of the preference for certain traits. Even though durable, rot-resistant poles can be made from *R. pseudoacacia*, there was still more of a preference for *Eucalyptus* species that produce straighter wood more suited to poles in general (Chikowore *et al*., 2021b). Smaller trees from suckers also form dense thickets with large thorns that make harvesting this tree difficult and therefore limits commercial utilisation (Chikowore *et al*., 2021b). The study by Chikowore (2021b) therefore suggests that incomes generated from *R. pseudoacacia* utilisation by rural communities are insignificant. Humphrey (2019) reported that farmers in the area also find the tree undesirable and that it is spreading uncontrollably. Furthermore, there are no commercial plantations of *R. pseudoacacia* in the area and therefore it is assumed that there will be no conflicts of interest regarding the control of *R. pseudoacacia* in the grassland biome of South Africa (Chikowore *et al*., 2021b).

6.3. Pre-release surveys

The presence of insects associated with *R. pseudoacacia* in South Africa was investigated. Previous research indicated that these insects were recorded in various regions, including North and South America, Europe, Southern Africa, Asia, and Australia (Sheppard *et al*., 2006). To determine their presence in South Africa, pre-release surveys were conducted in established areas where the tree was spreading. Over a period of three years, no insects known to be associated with the species in USA or Europe were found during the annual surveys.

The second objective of this thesis, described in chapter 2, aimed to assess the condition of *R. pseudoacacia* trees in the selected study area and to identify the primary mode of reproduction used by the tree in order to target it for biological control. To achieve this, various factors were evaluated, including leaf examination, population densities, soil seed bank and predation of seeds by birds and mammals. In general, trees located in or near pristine grassland or farmland were found to be undamaged. However, those in mixed stands with other trees in urban areas exhibited signs of damage from generalist insects. It is assumed that the absence of natural enemies in protected areas, grasslands, and farmlands allowed these trees to thrive and become invasive - *i.e.* enemy release hypothesis. The study revealed that vegetative reproduction through suckering was the primary mode of spread for *R. pseudoacacia* in South Africa, rather than sexual reproduction via seeds. Consequently, the exclusion of seed-feeding insects as a priority for biological control was recommended, as controlling seed dispersal might not effectively curb the tree's expansion. Instead, the focus should be on controlling leaf growth and health to influence the overall well-being of the tree and slow down its spread.

The emphasis on population density surveys and the impact of mechanical damage on stand growth provided valuable insights. Mechanical damage promoted denser stands and faster expansion, underscoring the need for management strategies to prevent such damage, such as controlled cutting or pruning. Therefore, this pre-release study significantly contributed to understanding the insect associations, reproduction modes, and spread dynamics of *R. pseudoacacia* in South Africa. By excluding seed-feeding insects as a priority for biological control and emphasizing leaf-targeting measures, future studies can develop more targeted and effective control measures, especially in areas where the tree poses a threat to protected ecosystems, grasslands, and farmlands. Understanding the dispersal mechanisms and other ecological interactions will be crucial in developing successful management strategies for *R. pseudoacacia* in South Africa.

The practical implications of the findings from the pre-release surveys and assessments of *R. pseudoacacia* in South Africa have substantial significance for informing the selection of potential biological control agents. The absence of insects associated with the species in the USA or Europe, despite the tree's widespread distribution in South Africa, highlights the need for a region-specific approach in choosing biological control agents. The emphasis on assessing the condition of *R. pseudoacacia* trees, particularly in urban areas where signs of damage from generalist insects were observed, underscores the importance of understanding the ecological dynamics contributing to the tree's invasive nature. The identification of vegetative reproduction through suckering as the primary mode of spread informs a targeted strategy, suggesting that prioritizing control measures against seed-feeding insects may not be as effective. The insights gained from population density surveys and the impact of mechanical damage emphasize the necessity for management strategies like controlled cutting or pruning to prevent denser stands and faster expansion. Overall, the study advocates for a nuanced and context-specific approach, excluding seed-feeding insects as a priority for biological control and emphasizing leaf-targeting measures, offering practical guidance for developing effective control strategies in areas where *R. pseudoacacia* poses a threat to protected ecosystems, grasslands, and farmlands. Understanding these ecological interactions is deemed crucial for the successful management of *R. pseudoacacia* in South Africa, adding practical value to future studies and control efforts.

6.4. Further insect prioritization by modelling climatic suitability

To further prioritize the most suitable biological control agent for the control of *R. pseudoacacia*, climate-matching models were used. The success of a control agent is largely dependent on its ability to establish and multiply in the introduced region, which is based on environmental factors and climate

(Wapshere, 1983). According to Lovell *et al*. (2004), insect survival, reproduction, and development are directly influenced by climatic factors such as precipitation, temperature, and humidity. These factors influence the distribution, establishment, and abundance of insects introduced as biological control agents to new regions (Cowie *et al*., 2016). If potential biological control agents are released in areas climatically matched to their native range, they are more likely to establish, proliferate, and control the intended weed compared to those that are incompatible (Wapshere, 1983).

In Chapter 4, insects prioritized in Chapter 3 were modelled using MaxEnt and CLIMEX-MCR. Predictions based on these models are not always perfect, but when interpreted correctly they can be valuable tools in biological control (Trethowan *et al*., 2011). The MaxEnt models overlaid climate variables from the insects' native range (USA) and invaded range (Europe) over South Africa to predict whether these insects were likely to survive and establish based on similar climate variables and to identify ideal release sites if they prove to be successful biological control candidates. All three insects had models predicting some suitability in South Africa, with *O. dorsalis*, the leaf-mining beetle, having the highest predicted suitability coverage over the eastern side of the country, albeit with the most uncertainty due to extrapolation. *Obolodiplosis robiniae* and *M. robiniella* had similar suitability predictions, covering a smaller area within the mountainous grasslands in the eastern Free State, with less extrapolation, making them more favourable as biological control agents for establishment in South Africa. CLIMEX-MCR models, overlaying South African climate over Northern America and Europe where these insects are found, indicated a perfect climate match, predicting that all three insects are capable of establishing and surviving in South Africa based on geographical data (Kriticos *et al*., 2021).

These models confirm that the insects prioritized in the previous chapters are good candidates as biological control agents, with *O. robiniae* and *M. robiniella* being slightly more favourable. Both insects have successfully escaped their native range (USA) and established in Europe, Asia, and the Pacific, with greater abundance in these invaded ranges, according to data from citizen science platforms and personal observations (Martin pers comm). In contrast, *O. dorsalis* has not been observed outside its native range, leading to uncertainty about its ability to survive and spread in a novel environment (Mally *et al*., 2021). The use of climate-matching models to assess the suitability of potential biological control agents is a valuable approach. By considering climatic factors and environmental conditions, the models provide essential insights into the likelihood of establishment and effectiveness of the selected insects in controlling *R. pseudoacacia*. However, it is essential to acknowledge that these models are preliminary steps, and further studies and testing are necessary to confirm the effectiveness and environmental impact of these insects as biological control agents in South Africa.

6.5. Host-specificity without laboratory trials

Due to the Covid-19 pandemic, fieldwork for this study was cancelled, and importing insects was unsuccessful. To further prioritize a biological control agent for *R. pseudoacacia*, three insects were examined, and it was found that there is a possibility of releasing *O. robiniae* without conducting host-specificity tests. Thorough literature investigations were conducted to explore the phylogenetic connections within the Papilionoideae Subfamily, to which *R. pseudoacacia* belongs. These studies revealed that no other species within the Robinia genus exists in South Africa. This scarcity of related species within the genus reinforces the safety of considering a genus-specific biological control candidate. Additionally, from the 353 species found in the same clade as *R. pseudoacacia*, 24 species are found in South Africa. None of them share any morphological similarities, reducing the likelihood of crossover to other plants. *Obolodiplosis robiniae* belongs to the Family Cecidomyiidae, which is known for its host-specificity. Literature and field trials support the fact that *O. robiniae* has not been found on other Fabaceae species and is highly specific to *R. pseudoacacia*. Furthermore, *R. pseudoacacia* is widespread across Europe where it is economically important, and *O. robiniae* is considered a pest that escaped its native range with the tree. However, there is no evidence that *O. robiniae* is a pest on other economically important plants related to *R. pseudoacacia*, making it unlikely to cross over to commercial or native plants in South Africa.

Through extensive literature reviews and field data, it is suggested that *O. robiniae* will be safe to be released as a biological control agent on *R. pseudoacacia* without conducting traditional host-specificity tests in quarantine conditions. The study provides a logical approach to evaluating the potential risks associated with releasing *O. robiniae* and compensates for the limitations imposed by the pandemic-related challenges. However, it is important to continue monitoring the introduced agent's behaviour and impact on non-target species after its release to confirm the accuracy of the predictions.

The study encountered several limitations, primarily arising from the COVID-19 pandemic, which resulted in the cancellation of fieldwork and the unsuccessful importation of insects. This constraint hindered the ability to conduct traditional host-specificity tests in quarantine conditions, raising potential concerns about the safety of releasing the chosen biological control agent, *O. robiniae*, without such tests. While the study employed thorough literature investigations to explore phylogenetic connections and establish the scarcity of related species within the *Robinia* genus in South Africa, it acknowledged the inherent uncertainties associated with relying solely on these findings. The absence of evidence that *O. robiniae* is a pest on other economically important plants related to *R. pseudoacacia* added a degree of assurance, but the lack of host-specificity trials introduced an element of risk. The study proposed a logical risk evaluation approach compensating for pandemic-related challenges but emphasized the ongoing need for post-release monitoring to verify the accuracy of predictions and assess any unforeseen impacts on non-target species. Despite these limitations, the study provided a pragmatic framework for navigating challenges and making informed decisions under constrained circumstances.

6.6. Additional considerations

In Martin *et al*. (2019) “Addressing geographical bias: A review of *Robinia pseudoacacia* (black locust) in the Southern Hemisphere” the author drew attention to the limited knowledge available on the invasive behaviour of the tree species in the Southern hemisphere, despite notable invasions. This thesis contributes to the understanding of *R. pseudoacacia* in South Africa and the broader Southern Hemisphere. It provides novel insights into various aspects, including average tree demographics, tree/stand health, disturbance effects, seeds and seed banks, and interactions of mammals and herbivory in South Africa.

The study's success in prioritizing potential biological control agents can be attributed to the wealth of data (peer reviewed articles, grey literature and agricultural extension platforms) accessible from the USA and Europe, even though these data were not initially intended for biological control purposes, thus requiring cautious interpretation. Through the amalgamation of citizen science and literature, the study made inferences and determinations regarding the impact, specificity, and biologies of the insects associated with the tree species. This innovative trend is both exciting and promising, especially with the anticipation of even greater accessibility in the future due to the rapidly expanding data generation, capture, and availability, potentially aided by machine learning capabilities.

The study underscores the significance of data capturing through platforms like iNaturalist, which is relatively new in the realm of biological control. The successful integration of computer-generated models, online literature, and citizen science data, particularly from iNaturalist, proved highly advantageous for biological control efforts. This approach not only demonstrated financial productivity but also enhanced decision-making processes, even in situations where differentiating between closely related insect species was challenging. The use of prediction models further facilitated the identification of the most suitable insect candidates for successful establishment and reproduction in South Africa or other regions requiring biological control interventions.

The approach adopted in this study presents immense potential for application by other biological control practitioners worldwide. For instance, *Gleditsia triacanthos*, another invasive tree species in South Africa, could benefit from these developed protocols, given the abundance of associated insect herbivore data available from the USA and Europe. However, the feasibility of this approach for other weed species must be considered cautiously, as it might be limited to well-studied species in popular families, such as Fabaceae. While confidence in the safety of potential agents is often high, the risk-averse nature of release committees is expected to maintain the necessity of standard host-specificity testing.

Robinia pseudoacacia in the Southern Hemisphere, posing a risk in extrapolating findings. The reliance on data from non-target regions, primarily the USA and Europe, raises concerns about overlooking region-specific factors, potentially limiting the applicability of the study's conclusions to South Africa. The cautious interpretation of data not originally intended for biological control purposes, including citizen science and literature, suggests a risk of overlooking critical aspects specific to biological control needs. Challenges in species differentiation, particularly for closely related insects, introduce a risk of misidentification and potential errors in drawing conclusions. Additionally, the feasibility of the approach for other weed species is cautiously noted, implying a potential risk of misapplication. The study acknowledges the necessity of standard host-specificity testing, emphasizing a risk-averse approach to releasing biological control agents without thorough evaluation to mitigate potential negative impacts.

6.7. Conclusion

This study provides a general description regarding the prioritization of potential biological control agents for the management of *R. pseudoacacia*, an invasive tree in South Africa. The study emphasizes the importance of pre-release surveys to understand the insect associations, reproduction modes, and spread dynamics of *R. pseudoacacia*. By focusing on leaf-targeting measures and understanding the factors influencing population density and mechanical damage, more targeted and effective control measures can be developed. The study also highlights the significance of prioritizing potential biological control agents before conducting host-specificity tests. A desktop review and scoring system was used to identify the most promising insects associated with *R. pseudoacacia*. The economic and social importance of the target weed was also considered to determine the feasibility and acceptance of a biological control strategy. Citizen science was employed to gather additional information on the selected insects' availability and distribution. Climate-matching models were utilized to further prioritize the most suitable biological control agent. The models predicted varying levels of suitability for the selected insects in South Africa, with *O. robiniae* and *M. robiniella* showing more favourable suitability predictions than *O. dorsalis*. The distribution patterns of the insects, along with their established presence in other regions, support their potential as effective biological control agents.

Due to the Covid-19 pandemic, fieldwork and importing of insects were not possible. However, based on extensive literature studies on phylogenetic relationships and the absence of closely related species in South Africa, it was hypothesized that *O. robiniella* could potentially be released without conducting host-specificity tests. Therefore, the study provides a systematic approach to prioritizing potential biological control agents for *R. pseudoacacia*. The findings suggest that *O. robiniae* and *M. robiniella* are more favourable candidates, considering their suitability predictions, established presence in other regions, and absence of closely related species in South Africa. Further studies and testing are necessary to confirm the effectiveness and environmental impact of these insects as biological control agents.

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8. Appendices

Appendix 5.1. The three closest related economically important plant species and their pests (Emden *et al*., 1998; Soroka & Otani, 2011; EPPO, 2022; McNeill *et al.*, 2022)

Glycyrrhiza glabra **(liquorice)**	***Cicer arietinum*** **(chickpeas)**	***Medicago sativa*** **(alfalfa)**
Altica deserticola	*Acyrthosiphon pisum*	*Acyrthosiphon kondoi*
Lampides boeticus (pea blue butterfly)	*Agrotis ipsilon* (black cutworm, winter cutworm)	*Agrotis segetum*
Leveillula taurica (powdery mildew of cotton)	*Aphis craccivora* (cowpea aphid)	*Camptopus lateralis*
Myllocerus undecimpustulatus	*Catopsilia pomona*	*Chrysodeixis eriosoma*
	Chrotogonus trachypterus	*Elasmopalpus lignosellu*
	Danaus chrysippus	*Epichoristodes acerbella*
	Gryllotalpa africana	*Epiphyas postvittana*
	Gryllus bimaculatus	*Epitrix tuberis*
	Helicoverpa armigera (cotton bollworm, Australian bollworm)	*Frankliniella occidentalis*
	Liriomyza cicerina (chickpea leafminer)	*Gonioctena fornicate*

Spilostethus hospes	*Helicoverpa armigera*
Spodoptera exigua (beet armyworm)	*Hishimonus phycitis*
	Homalodisca vitripennis
	Hypera postic
	Liriomyza huidobrensis
	Liriomyza sativae
	Liriomyza trifolii
	Listronotus bonariensis
	Lobesia botrana
	Naupactus xanthographus
	Nysius huttoni
	Omophlus lepturoides
	Platynota stultana
	Popillia japonica
	Prodiplosis longifila
	Scirtothrips citri
	Spodoptera frugiperda

Spodoptera littoralis

Spodoptera litura

Spodoptera ornithogalli

Thrips angusticeps

Thrips imagines

Trogoderma granarium

Tuta absoluta

Appendix 5.2. Insects that are associated with plants that are closely related to *Robinia pseudoacacia* in order to determine whether insects associated with *Robinia pseudoacacia* are found feeding on other related plants (NSW, n.d; Wipfli *et al*., 1989; Boa & Lenne, 1996; Sileshi *et al*., 2000; Alzugaray, 2003; Sileshi *et al*., 2004; EPPO, 2012; Schmitt, 2015; Brian *et al*., 2019; ACIAR, 2020; CABI, 2023; Carlos *et al*., 2022; EPPO, 2023).

Plant species closely related to ***Robinia pseudoacacia***	Prominent insect herbivores
Robinia hispida	*Epargyreus clarus* (Silver-Spotted Skipper)
	Erynnis icelus (Dreamy Duskywing)
	Mellilla xanthometata (Orange-Wing)
	Dasylophia anguina (Black-Spotted Prominent)
	Ecdytolopha insiticiana (Locust Twig Borer Moth)
	Aeolothrips vittipennis (Thrips sp.)

	Neohydatothrips annulipes (Thrips sp.)
	Brochymena quadripustulatus (Four-Humped Stink Bug)
	Achryson surinamum (Long-Horned Beetle sp.)
	Amblycerus robiniae (Locust Seed Borer)
	Odontata dorsalis (Locust Leafminer)*
	Sumitrosis rosea (Leafminer Beetle sp.)
	Autochton cellus (Golden Banded-Skipper)
	Erynnis funeralis (Funereal Duskywing)
	Eurema mexicana (Mexican yellow)
Robinia neomexicana	*Appendiseta robiniae*
	Phellinus robiniae
	Epargyreus clarus (Silver-Spotted Skipper)
	Autochton cellus
	Cerotoma trifurcate (Bean Leaf Beetle)
	Disonycha punctigera (Flea beetle)
	Liriomyza trifoliearum (leaf-miner fly)
Securigera varia	*Colias eurytheme* (Orange Sulfur)
	Lycaenides melissa melissa (Melissa Blue)
	Erynnis baptisiae (Wild Indigo Duskywing)
	Adelphocoris limbatus (Alfalfa Plant Bug)

	Holcostethus limbolarius Marmorated (Brown Stink Bug)
	Melanoplus differentialis (Differential Grasshopper)
	Melanoplus femurrubrum (Red-legged Grasshopper)
Coronilla coronate	*Liriomyza bryoniae*
	Liriomyza cicerina
	Liriomyza congesta
	Phytoliriomyza variegata
	Aproaerema anthyllidella
	Leucoptera lotella
Coronilla scorpioides	*Liriomyza bryoniae*
	Liriomyza cicerina
	Liriomyza congesta
	Phytoliriomyza variegata
	Aproaerema anthyllidella
	Leucoptera lotella
Gliricidia sepium	*Aphis craccivora* (aphid)
	Ferrisia virgate (striped mealybug)
Sesbania sesban	*Odontotermes* spp (termites)
	Mesoplatys ochroptera
	Ootheca benningseni

Zonocerus variegatus

Megalurothrips sjostedti

Lipaleyrodes sp.

Anoplocnemis curvipes

Aphis fabae

Mylabris dicincta

Coryna mylabroides

Megalurothrips sjostedti

Haplothrips gowdeyi Franklin,

Sericothrips adolfifriderici

Haplothrips nigricornis

Eurytoma sp.

Pseudotorymus sp.

Agonoscelis pubescens

Anoplocnemis curvipes

Aphis fabae

Brachyplatys testudonigro

Coptosoma sp.

Ferrisia sp.

Graptostethus sp.

Hilda patruelis

Icerya purchasi

Leptocoris amicta

Lipaleyrodes sp.

Nezara viridula

Spilostethus sp.

Oxyrachis tarandus

Chrysolagria sp.

Euproctis rubricosta

Exosoma sp.

Lagria villosa

Medythia quaterna

Mesoplatys ochroptera

Ootheca sp.

Plusia orichalcea

Tetranychus sp.

Zonocerus variegatus

Alcidodes erythropterus

Megalurothrips sjostedti

Mylabris dicincta

	Acanthoscelides obtectus
	Sycophila sp.
Sesbania punicea	*Chinavia hilaris* (green stink bug)
	Pectinophora gossypiella (pink bollworm);
	Phakopsora pachyrhizi (soyabean rust)
	*Trichapion lativentre**
	*Rhyssomatus marginatus**
	*Neodiplogrammus quadrivittatus**
Hippocrepis glauca	*Thrips tabaci* (Onion Thrip)
	Zygaena ignifera
Hippocrepis comosa	*Polyommatus coridon* (chalk-hill blue)
	Polyommatus bellargus (Adonis Blue)
	Erynnis tages (Dingy Skipper)
Anthyllis hermanniae	*Xylella fastidiosa*
Anthyllis barba jovis	No data
Anthyllis terniflora	No data
Ornithopus pinnatus	*Penthaleus spp.* (blue oat mite)
	Halotydeus destructor (redlegged earth mite)
	Sminthurus viridis (lucerne flea)
	Listroderes costirostris obliquus (vegetable weevil)

	Helicoverpa punctigera (native budworm)
Ornithopus compressus	*Heliocoverpa* spp.
Tripodion tetraphyllum	No data
Lotus uliginosus	*Lygus* spp. *Calocoris norvegicus* *Helicoverpa armigera* *Walshia* spp. *Merophyas divulsana* *Dichomeris ammoxantha*
Lotus corniculatus	*Bruchophagus platypterus* *Epinotia aporema* *Adelphocoris lineolatus* *Lygus lineolaris* *Plagiognathus chrysanthemi* *Colias eurytheme* (Orange Sulfur)
Lotus subbliflorus	No data
Lotus macroccanus	No data
Coursetia caribaea	*Stator pruininus* *Meibomeus ptinoides*
Coursetia glabella	No data

Olneya tesota	*Aphis* spp. (Aphids)
	Thrips spp. (Thrips)
	Aleyrodidae (Whiteflies)
	Psyllidae (Plant lice)
Peteria thompsoniae	No data
Peteria scoparia	No data
Sesbania herbacea	*Mussidia nigrivenella* (cob borer)
	Colletotrichum truncatum
	Scirtothrips dorsalis
Lotus hamatus	No data
Lotus aboriginus	No data
Lotus pyranthus	No data
Lotus eriosolen	No data
Lotus arenarius	No data
Lotus purpureus	No data
Lotus rigidus	No data
Hosackia pinnata	No data
Hosackia stipularis	No data
Acmispon denticulauts	No data
Acmispon parviflorus	No data

Acmispon americanus	No data

Appendix 5.3. Sites in Germany and Switzerland that were examined for the presence of *Obolodiplosis robiniae* on neighbouring Fabaceae plants

Family/Sub Family	*Species*	Number of Sites	Number of galls containing *O. robiniae*
Faboideae	*Lotus corniculatus*	5	0
Faboideae	*Securigera varia*	3	0
Faboideae	*Medicago sativa*	7	0
Faboideae	*Medicago lupulina*	5	0
Faboideae	*Trifolium medium*	5	0
Faboideae	*Trifolium dubium*	1	0
Faboideae	*Trifolium repens*	5	0
Faboideae	*Trifolium sp.*	3	0
Faboideae	*Meliotus albus*	1	0
Faboideae	*Lathyrus linifolius*	1	0

8.1. Appendix References

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