DISSERTATION SUBMISSION

Demographics of alien willows in the Grassland Biome of South Africa

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Submitted in fulfilment of the requirements in respect of the Masters degree in Botany in the Department of Plant Sciences in the Faculty of Natural and Agricultural Sciences at the University of the Free State.

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Declaration

I , Tapiwanashe Mashamba, declare that the Master's Degree research dissertation or interrelated, publishable manuscripts/published articles, or coursework Master's Degree mini-dissertation that I herewith submit for the Master's Degree qualification in Botany at the University of the Free State is my independent work, and that I have not previously submitted it for a qualification at another institution of higher education.

29 November 2024

Student's Signature Date

Department of Plant Sciences
Natural and Agricultural Sciences

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A very special thank you goes to my family. Despite my long times away from home I am sure your prayers were the extra fuel I needed to make it to the finish line. This degree is dedicated to every one of you. The sacrifices you made on a daily basis since 2016 have led to this achievement. With this achievement the ceiling keeps getting higher and with this I promise to keep reaching for new ways to make you proud.

"It is a rough road that leads to the heights of greatness."- Lucius Annaeus Seneca

Abstract

Invasive alien species present a critical threat to biodiversity, ecosystem services, and agricultural productivity worldwide. This study examines the distribution, demographic dynamics, and ecological impacts of *Salix babylonica* L. (weeping willow) and *Salix fragilis* L. (crack willow) within the Grassland biome of the Eastern Free State, South Africa. These invasive species, introduced for ornamental and functional purposes, have shown extensive colonization, particularly in riparian zones, where they outcompete native vegetation and alter hydrological processes.

The research combines historical and contemporary data sources to evaluate changes in the population structure and spatial distribution of these species over time. Using aerial photographs from 1984 and 2023, supplemented with roadside surveys and Google Street View (GSV) analysis, the study provides an assessment of the species' demographics. Additionally, field-based verification of GSV-derived data validates its utility as a cost-effective tool for mapping and monitoring invasive alien plants.

The study confirms that *S. babylonica* and *S. fragilis* remain prevalent along watercourses, roadsides, and disturbed areas, with *S. babylonica* exhibiting a broader distribution along roadsides with approximately 50% of the sites having a population size of 1-5 trees. Although *S. fragilis* is not as widely distributed as *S. babylonica*, it dominates the areas in which it is found, forming pure stands; 56% of the sites surveyed consisted of 6 or more trees. Gender analyses of *S. babylonica* populations reveal a predominance of female trees which suggests that its propagation is mainly asexual.

The demographic study done through road surveys and GSV reveals limited recruitment of *S. babylonica* in recent decades, suggesting a potential decline in its invasive potential with almost 80% of the population consisting of adult or dying trees. In contrast, *S. fragilis* populations

display a continuous expansion with healthy recruitment, with 44% of the trees recorded as seedlings, young trees, or mature trees. This expansion indicates that *S. fragilis* is now more invasive than *S. babylonica*, which increases the need for a tailored management strategy to address this invasive species.

The research also investigates interactions between invasive willows and invertebrate communities. Surveys at selected sites document the presence of 348 individual insects and one arachnid, representing 21 families, with 14 species associated with both willow species. Another finding was the presence of crown galls on 27.5% on the *S. babylonica* population surveyed. Invasive willows provide habitat and resources for certain insect populations. Some of these insects, specifically *Plagiosterna discolor*, *Tuberolachnus salignus* and *Helionidia quadrimaculata,* along with gall-inducing bacteria (potentially *Agrobacterium tumefaciens*), recorded within the *S. babylonica* populations could be resulting in the decline of this invasive species.

This study explored the use of ArcGIS and GSV, to assess invasive tree distributions and potential of these tools to monitor the spread of invasive trees over time. The comparison of historical and current data reveals significant efficiency of GSV as a monitoring tool, offering a cheap and quick approach to invasive species management in resource-limited settings.

Policy implications of the findings are discussed with reference to South African legislation, including the Conservation of Agricultural Resources Act (CARA) of 1983 and the National Environmental Management: Biodiversity Act (NEMBA). *Salix babylonica* and *S. fragilis* have been classified as Category 2 invaders under CARA but are not found under the NEMBA Act. This may result in challenges in effectively controlling *S. babylonica* and *S. fragilis.*

This dissertation contributes to the field of biological invasions by providing an updated assessment of invasive willows in the Grassland biome, thereby offering insights for land managers, conservationists, and policymakers. The study concludes by emphasizing the importance of integrating historical data and modern geospatial tools, as an informative approach to develop effective management solutions for invasive alien plants. The methodological framework and findings presented herein offer valuable lessons for addressing invasive species challenges in similar ecological contexts globally.

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CHAPTER 1

LITERATURE REVIEW AND BACKGROUND INFORMATION

Invasive alien species

Biological invasions worsen every decade, posing severe environmental risks (Ehrenfeld, 2010; McGeoch et al., 2015; Powell et al., 2013). Biological invasions can happen in both terrestrial and aquatic habitats (Stone et al., 2018). The movement of a species into a new environment is primarily driven by globalization (Kueffer, 2017), though factors such as animals, wind, and water may also contribute to its dispersal (Lamberti-Raverot et al., 2019). When these species move to new places, they can pose a significant threat to the existing diversity of plants and animals, and ecological balances, such as how nutrients move through the ecosystem (Ehrenfeld, 2010; Powell et al., 2013).

Before an alien species transitions into an invasive alien species, it is typically classified as a naturalised alien species (Richardson and Rejmánek, 2011; Figure 1.1). A naturalized alien species remains restricted in its spread and has not yet established itself in natural ecosystems, semi-natural ecosystems, or human-modified disturbance zones, whereas an invasive alien species has not only expanded into these areas but also exerts a significant ecological impact by altering community dynamics, competing with native species, or disrupting ecosystem processes (Richardson et al., 2000). It is important to note that not all naturalised alien species inevitably become invasive (Richardson et al., 2000).

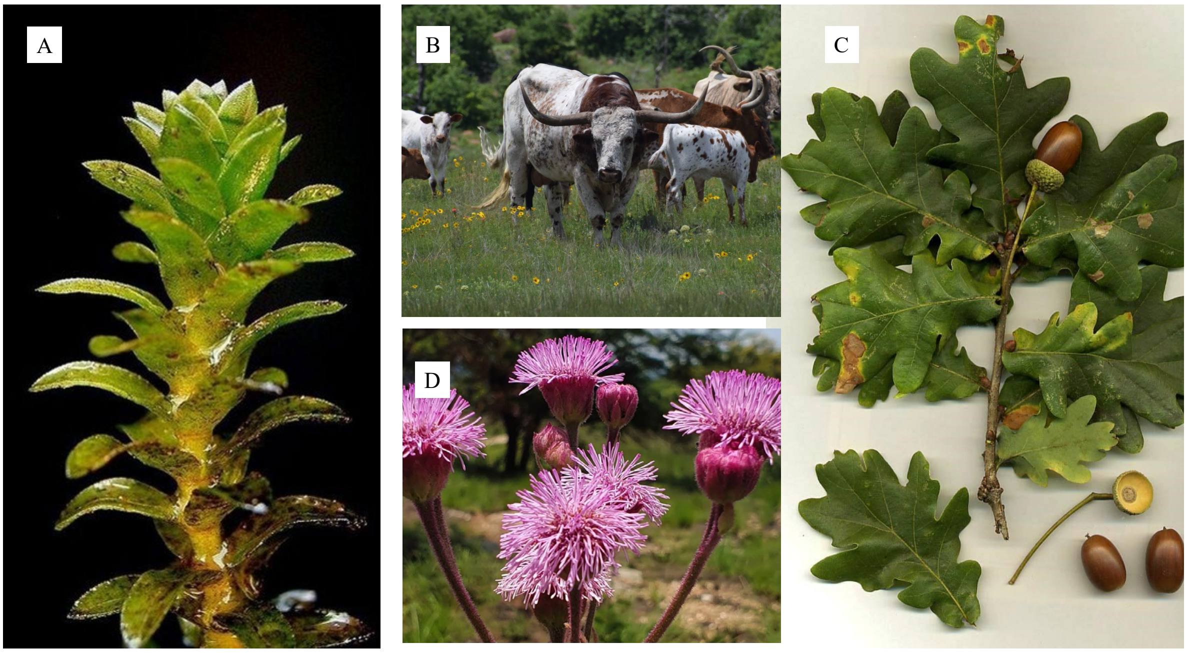

Figure 1.1: Examples of species in each stage of the continuum of the naturalisation of alien species to becoming invasive. (A) Introduction- *Elodia candensis* Michx (American water weed, Hydrocharitaceae) - recorded in aquarium trade in South Africa (Martin and Coetzee, 2011) but not escaped. (B) Establishment- *Bos taurus* L. (domestic cows) - introduced along the Atlantic seaboard and the Limpopo River Basin (Sadr, 2015). (C) Naturalisation- *Quercus robur L.* (English oak, Fagaceae) - this is the most common oak tree found in South Africa, and it is non-indigenous, planted by early European settlers in the Cape region (Gildenhuys et al., 2024). (D) Invasion- *Campuloclinium macrocephalum* Less. (Pompom weed, Asteraceae) - native to South America (Argentina and Brazil), Central America and Mexico, is now a renowned invasive species in South Africa where it was probably introduced as an ornamental plant (Henderson, 2001).

Invasive alien plants

Plants have been moved around the world to satisfy basic human needs (Mack and Lonsdale, 2001). This includes crop plants such as spices, tea, coffee, and cotton, being sought after for their economic value, and other plants being moved for medicinal and ornamental purposes (Mack, 1999; Mack and Lonsdale, 2001; Zengeya et al., 2017). Trees have also been moved for lumber, soil stabilisation and other purposes (Mack and Lonsdale, 2001). Some of these species may escape and become problematic. According to Lowe et al. (2000), Invasive Alien Plants (IAPs) are the most damaging invasive species. The world economy loses an estimated US$300 billion per year worldwide as a result of alien invasive plants (Pimentel et al., 2000;

Genovesi, 2011). Invasive alien species disrupt agriculture, alter ecosystems, and interfere with infrastructure (Canyon et al., 2002; Pratt et al., 2013). These impacts also extend beyond financial costs, affecting biodiversity, soil erosion, and water resources (Canyon et al., 2002). One of the major impacts is on water resources as invasive plants can damage water infrastructure, such as pipes, canals, and dams (Crookes et al., 2020). Their roots can penetrate and clog pipes, and their growth can interfere with the operation of water supply system (Crookes et al., 2020). The need for repairing and maintaining this infrastructure increases, affecting the reliability of water services (van Wilgen et al., 2008). During floods, senescent trees like *Salix fragilis* L. (Salicaceae) (crack willow) can drop large branches or trunks into waterways and a build-up of material behind these blockages can destroy bridges (Zukowski and Gawne, 2006). Many IAPs, such as *Salix fragilis* and *Eucalyptus* species, have higher water consumption rates than native plants (Scott et al., 1999). This excessive water uptake reduces the availability of water for other uses, including agriculture, drinking water supplies, and natural ecosystems (Le Maitre et al., 2002).

Invasive alien plants may become dominant in the landscape or waterways due to a number of attributes or plant traits (Li and Shen, 2020; Rai and Singh, 2020) including: fast growth, fast reproduction, high dispersal ability, tolerance to a wide range of environmental conditions (ecological competence), and the ability to form large and long-lasting soil seed banks (Foxcroft et al., 2011). Once in a new environment, an alien plant propagule needs to grow and reproduce successfully, and subsequent naturalization is only possible with the successful production of viable seeds or with plants spreading by asexual reproduction (Richardson et al., 2000). Other factors that can influence the naturalization of IAPs in a new environment include the availability of resources, suitable conditions for germination and growth, and the availability of potential dispersers of the plants (Foxcroft et al., 2011). Multiple theories have been suggested to explain the success of invasive species. The enemy release theory proposes that the success of an IAP is due to the lack of enemies in the new environment, such as herbivores and diseases (Keane and Crawley, 2002; Rai, 2015). Another theory is the empty niche hypothesis which suggests invasives and natives live in different niches, which is influenced by differences in phylogeny and traits, and that competition between invasives and natives is weak (Chesson, 2000; Rundel et al., 2014). This means the invasive species are able outcompete the native species by exploiting resources not used by or essential to the native species (Rundel et al., 2014).

It is worth noting that that some invasive species, in addition to their negative impacts, may also have some beneficial attributes (Zengeya et al., 2017). These are referred to as conflict-of-interest species (Zengeya et al., 2017). In many areas of South Africa traditional medicine markets and shops have been found to sell alien plant species or parts thereof (Byrne et al., 2017). Many invasive plants in South Africa have substantial economic value. For instance, species like *Eucalyptus* and *Acacia* are widely used in the timber and paper industries. The economic benefits derived from these species include job creation, revenue generation from wood products, and contributions to the local and national economy (Crookes et al., 2020). These industries are vital to the livelihoods of many South Africans, particularly in rural areas where job opportunities may be scarce (Zengeya et al., 2017). In rural South Africa, where access to electricity and modern energy sources may be limited, invasive species like *Acacia* and *Eucalyptus* are important sources of fuelwood and charcoal (Zengeya et al., 2017). These resources are crucial for cooking and heating, especially in impoverished communities. The availability of these invasive species reduces the pressure on indigenous forests and other native vegetation that might otherwise be overharvested (Semenya et al., 2012). Other than economic impacts, certain invasive plant species hold cultural significance in South Africa. *Acacia* species, though invasive, are sometimes utilized in traditional medicine, rituals, and as sources of firewood (Zengeya et al., 2017). In communities where traditional practices are an integral part of life, these species are valued not only for their practical uses but also for their role in preserving cultural heritage (Zengeya et al., 2017).

Impacts of alien plant invasions in South Africa

According to the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES), IAPs are one of the main causes of biodiversity loss (Diaz et al., 2019). Invasive alien plants change the way ecosystems work by changing the physical and chemical characteristics of ecosystems, such as soil composition and hydrology (Psyek et al., 2020). Invasive alien plants can negatively impact ecosystem services such as water filtration, carbon sequestration, and pollination, which can have secondary effects on other organisms (Vila et al., 2010). In addition, IAPs have negative effects on the ability of catchment areas to store and release water as well as its quality. Some *Salix* species have deep tap roots that enable them to absorb lots of groundwater (Henderson, 1991), taking away water from the native plants. IAPs also cause economic impacts by increasing the risk of damage to infrastructure. For example, cheatgrass, *Bromus tectorum* L. (Poaceae), increases the risk of wildfires by creating a continuous fuel source in western America (Pyke et al, 2016), and the giant reed, *Arundo donax* L. (Poaceae), increases the risk of flooding in northern America (Spencer et al., 2013). Invasive alien plants can also spread pests to a new area. The kudzu bug, *Megacopta cribraria* Fabricius. (Plataspidae) is an invasive stink bug that feeds on the invasive vine kudzu, *Pueraria montana* (Lour.) Merr. (Fabaceae), which is native to Japan but has spread widely in South Africa (Burrows, 1989). The kudzu bug can cause significant damage to soybean crops (Grant et al., 2014). Given these examples is necessary to see how much of South Africa is affected and in what ways alien plants have invaded different biomes even before they become invasive.

It is estimated that 70% of South Africa's land is covered by vegetation from Grassland, Savanna, Thicket, and Karroid shrubland habitats (O'Connor and van Wilgen, 2020). There are significant changes in the composition and distribution of IAPs in South Africa within these habitats (Henderson and Wilson, 2017). This vegetation supports both domestic animals (approximately 12.8 million cattle; 22.5 million sheep; 5.4 million goats) and natural herbivores (Department of Agriculture, Forestry and Fisheries, 2019). Within this diversity of flora, hundreds of alien plant species can be found spread amongst the native plants in each South African biome (van Wilgen and Wilson, 2018). The Nama Karoo Biome harbours an estimated 129 alien species, while the Savanna Biome has 404 alien species (van Wilgen and Wilson, 2018). Numerous alien species are still limited to areas that have been altered by urbanization, forestry plantations, and crop cultivation. The biomes most affected by IAPs are those with more water (Grassland, Savanna, Indian Ocean Coastal Belt), as well as riparian rather than dryland habitats within each biome (O'Connor and van Wilgen, 2020).

The primary ecological service offered by 70% of South Africa's land is the production of animal products through rangelands. It is estimated that livestock production has been reduced by ZAR 340 million annually due to current plant invasion levels (De Lange and van Wilgen 2010; O'Connor and van Wilgen, 2020). In addition to providing fuel wood, native medicines, wildlife-based tourism, pollination services, and the biodiversity that supports all of these, rangelands are essential for the delivery of water services. The anticipated annual value of the water resources given by the higher rainfall areas of the Grassland Biome in the absence of invaders was ZAR 50 billion per year; however, invasions in 2010 caused this value to drop to ZAR 48.9 billion (De Lange and van Wilgen, 2010). The annual value of biodiversity-based services from all terrestrial biomes through non-use of resources such as tourism was estimated at ZAR 21.7 billion per year due to the current levels of invasion (De Lange and van Wilgen, 2010). These services are estimated to be valued as high as ZAR 22.1 billion per year in the absence of invasion by alien plant species (De Lange and van Wilgen, 2010).

Invasive alien trees in South Africa

Southern Africa has been invaded by many more invasive alien tree species compared to other regions of the world (Richardson and Rejmánek, 2011). Two-hundred and forty (32%) of the 759 naturalized alien plant species in South Africa are trees, with the 200 of these being invasive species (Richardson et al*.,* 2020). These trees were introduced through agroforestry (Richardson and Rejmánek, 2011; O' Connor and van Wilgen, 2020), or horticulture since 1652, when companies like Company Gardens in Cape Town established their trade (Richardson et al., 2020). The most common invasive plant species include the black wattle and Jacaranda. Black wattle (*Acacia mearnsii* De Wild., Fabaceae) from Australia, brought to South Africa in the late 1800s and it is now found all over the country, especially in the Eastern Cape and KwaZulu-Natal areas, where it has become a major invasive species (Le Roux et al., 2018). *Jacaranda mimosifolia* D.Don (Bignoniaceae) is also a common invasive tree species. Originally from South America, *Jacaranda mimosifolia* was introduced to South Africa as an ornamental tree in the late 1800s and has since become naturalized in many urban areas of the country (Richardson et al., 2020). Over time, the South African horticultural industry has grown, and many of the most common invasive plants in South Africa, especially near towns, kept spreading through human influence because they looked attractive (Zengeya et al., 2017). The invasive representatives of the *Acacia*, *Casuarina*, *Eucalyptus*, *Pinus*, *Prosopis*, and *Schinus* genera have been thoroughly examined in South Africa. Knowledge of these taxa's invasions has greatly advanced our knowledge of tree invasions worldwide (Rundel et al., 2014).

Some ecosystem types in South Africa are more vulnerable to invasion and alteration by alien trees because invaders have extensive propagule pressure and lengthy residence periods resulting from several importation and extensive plantings over the past century and a half (Richardson et al*.,* 2020). South Africa's riparian environments have been extensively overrun by alien trees (Richardson et al., 2020). Flood-related disruption and propagule distribution along rivers are the main causes of these invasions (Richardson et al., 2020). These invasions tend to reinforce themselves, as the affected ecosystems offer ample habitat for the establishment of seedlings and detached plant parts (Galatowitsch and Richardson, 2005; Holmes et al., 2005). In South Africa, widespread invasions are particularly prevalent in wetter regions, including perennial rivers extending through the arid Karoo, Savanna, and Grassland biomes. These invasions are predominantly composed of tree species from genera such as *Acacia*, *Eucalyptus*, *Populus*, and *Salix* (Richardson et al., 2020). Among these, *Salix babylonica* L. and *Salix fragilis* L. (both in the family Salicaceae) are considered dominant invasive species within the river systems of the Grassland biome (Henderson, 1991, 2007).

***Salix* species**

Salix is a genus from the Salicaceae family, commonly known as willow trees. The family Salicaceae also includes the *Populus* genus, native to the Northern Hemisphere. According to the Flora of North America, there are about 450 species of willow worldwide (Argus, 2010), most of which are native to the temperate regions of North America, Europe, and Asia (Dickmann and Kuzovkina, 2014) with a notable presence in the arctic zones, as well as extending into subtropical and tropical regions along montane ranges. The centre of diversity for *Salix* is in China, which harbors nearly 200 native species, while North America supports 113 species (Wagner et al., 2021). Although willows have a broad native distribution, several species have been introduced to new regions and have subsequently become invasive. This is particularly evident in countries such as Australia, New Zealand, and South Africa, where species like *S. cinerea*, *S. fragilis*, and *S. babylonica* exhibit aggressive growth and pose ecological challenges by outcompeting native vegetation (Caron et al., 2014).

Salix babylonica

Salix babylonica, also known as the weeping willow, is native to northern China (Henderson, 2001). The tree was introduced to other parts of the world, including Europe and southern Africa, as an ornamental plant (Henderson, 2001). It is known for its long, pendulous branches and is often planted near water features. In China, *S. babylonica* is typically found growing along riverbanks, lakeshores, and other wetland areas (Orwa, 2009), and is a popular ornamental tree in gardens, often featured in traditional Chinese paintings and literature (Bennett and Meredith, 2004; Figure 1.3). The weeping willow is also widely cultivated in China for various purposes, such as erosion control, landscaping (Baker, 2009), and as a source of biomass for fuel and papermaking (Baker, 2009).

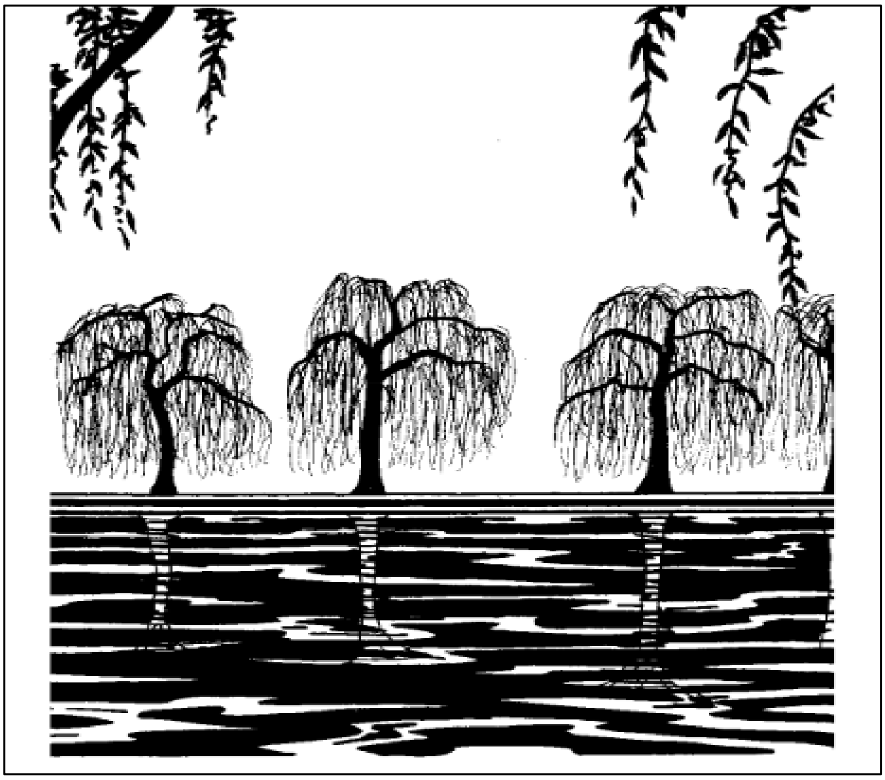

Figure 1.3: Artistic depiction of *Salix babylonica* in Chinese landscape art (Bennett and Meredith, 2004).

Salix babylonica is a broad-headed, large tree with flexile hanging greenish to brown branches, growing up to 20–25 meters tall (Henderson, 2001). The leaves are alternate and spirally arranged, narrow, light green, 4–16 cm long and 0.5–2 cm broad, which turn gold–yellow in autumn. The flowers are arranged in catkins (Figure 1.4) produced early in the spring with the male and female catkins found on separate trees (Henderson, 2001). Due to hanging branches, this tree forms dense patches of tall and robust canopies (up to 6 meters) along river sides and dam edges (Henderson, 2001). The identification of *S. babylonica* is easy and does not require close *in situ* observation because of its pendulous branches (Marchenko and Kuzovkina, 2023; Figure 1.5).

Figure 1.4: Line drawing of female single flower (A) found as female inflorescence, sessile, in catkins (B). The fruit is a greenish, sessile capsule (C) in catkins. Extracted from Henderson (2001)

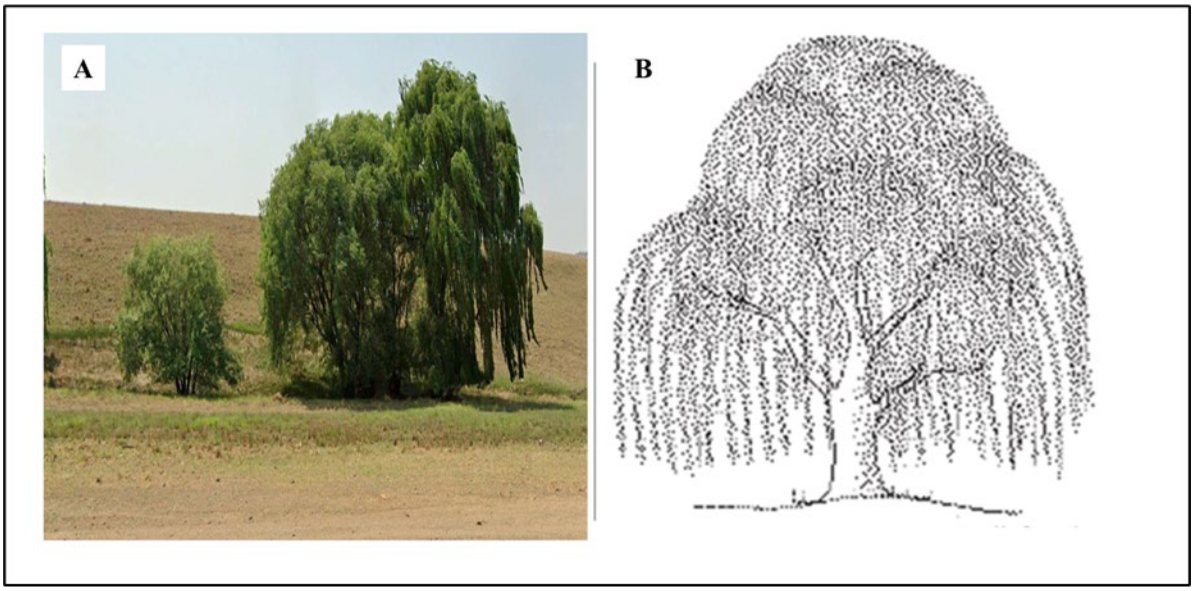

Figure 1.5: (A) *Salix babylonica* along roadside in Grasslands in the Free state province. (Photograph: T. Mashamba). (B) Line drawing of *Salix babylonica,* Extracted from Henderson (2001).

Salix fragilis

Salix fragilis, also known as crack willow, is a typical tree of riversides, stream banks, and other permanently wet sites (Kennedy et al., 2003). It is an adaptable tree with a capacity to disperse over wide areas along watercourses by vegetative reproduction (Henderson, 1991). The trees are often multi-trunked with an irregular and often leaning crown (Figure 1.6) (Henderson, 2020). *Salix fragilis* is native to Western Europe and Asia (Henderson, 1991). It has a history of invasiveness where introduced, including Chilean Patagonia, Patagonia, Argentina and South Africa (Henderson, 1991; Budde et al., 2011; Lewerentz et al., 2019). In South Africa, it was found to be naturalized along watercourses, indicating a potential introduction for ornamental or erosion control purposes by stabilising banks and dykes (Henderson, 1991).

Salix fragilis is a medium to large deciduous tree that grows rapidly, reaching heights of up to 15 meters, with trunks up to 1 meter in diameter (Henderson, 2001). Its bark is dark grey-brown and becomes coarsely fissured as the tree matures. The lance-shaped leaves are bright green with finely serrated edges (Henderson, 2001). Initially, in spring, the leaves are very finely hairy but soon become smooth and hairless (Henderson, 2001). The tree produces flowers in the form of catkins (Figure 1.7) during early spring and is dioecious, meaning male and female catkins occur on separate trees (Henderson, 2001). By late spring, its fruit capsules release numerous small, cotton-tufted seeds that are easily dispersed by wind and water, germinating immediately upon contacting soil (Henderson, 2001).

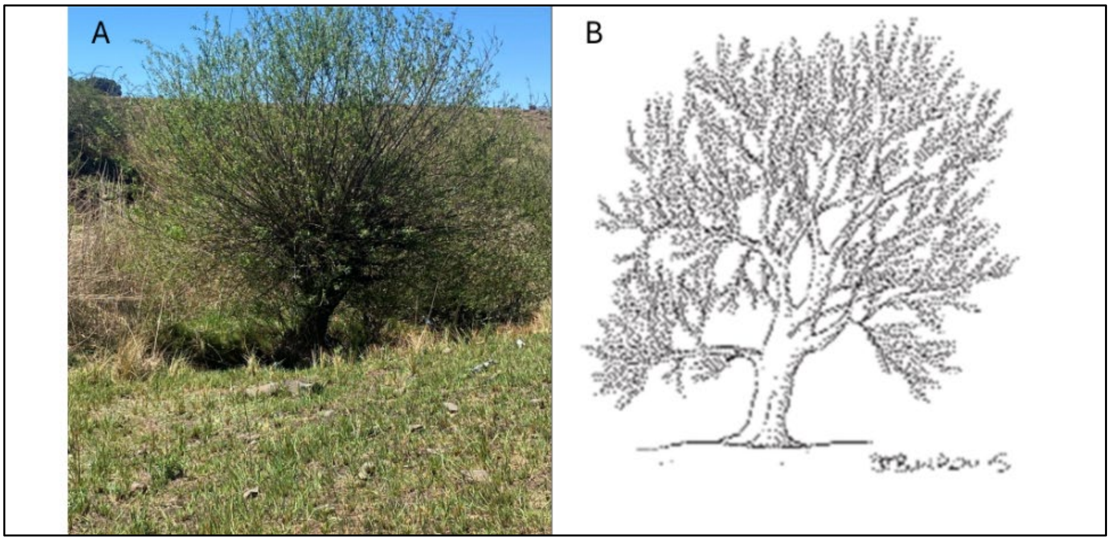

Figure 1.6: *Salix fragilis* along roadside in Grasslands (Photograph: T Mashamba) (A); Line drawing of *Salix fragilis* (B) Extracted from Henderson (2001).

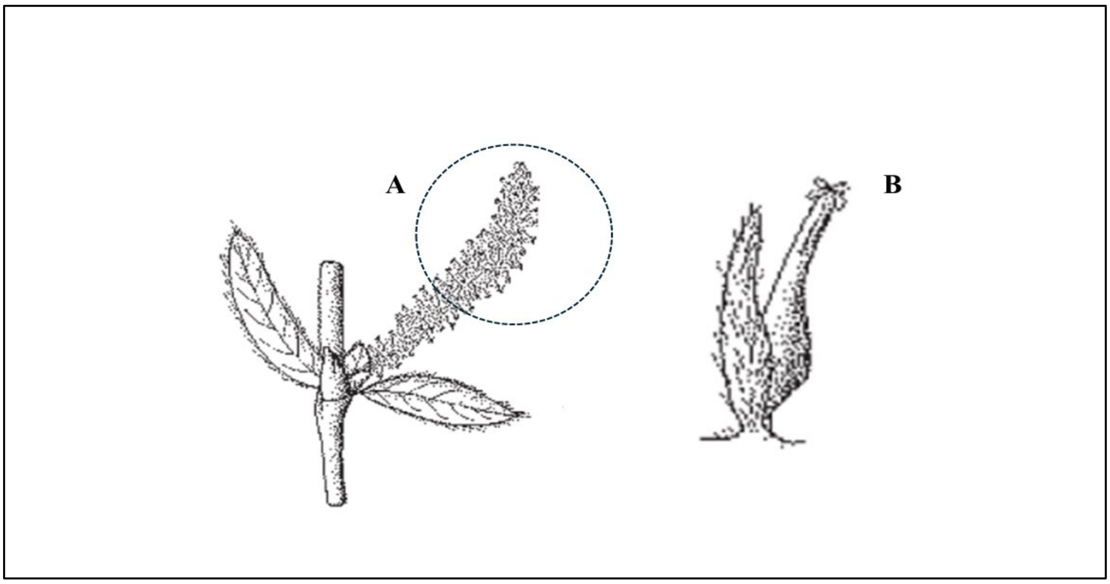

Figure 1.7: Line drawing found as female inflorescence, sessile, in catkins (A). Line drawing of female single flower of *Salix fragilis* (B) Extracted from Henderson (2001).

Global impacts of invasive *Salix babylonica* and *Salix fragilis*

Negative impacts

Salix babylonica and *Salix fragilis* both dominate the vegetation next to water sources. This is because both species are highly adaptable to waterlogging (Zhong et al., 2013) and have high water requirements. They can deplete water sources, particularly in areas with limited water availability. This can lead to competition with other plants and affect local ecosystems, especially in regions prone to drought (Zhong et al., 2013). Without changes in water management in native areas, exotic *Salix* species will dominate the riparian zones (Kennedy et al., 2003). While willows are often planted for greening and environmental bioremediation in mining subsidence areas in countries outside their native range to control erosion (Baker, 2009), certain species such as *S. fragilis* can exacerbate erosion due to their aggressive root systems, to the extent that they can alter the natural courses of rivers (Purtle et al., 2001). Their roots can penetrate deep into the soil and weaken riverbank structures, potentially leading to increased sedimentation and habitat loss for aquatic species (Purtle et al., 2001).

Positive impacts

Despite their invasive nature, *S. babylonica* and *S. fragilis* remain valued for their aesthetic qualities (Henderson, 1991; Popova and Kaleva, 2015). *Salix babylonica* and *S. fragilis* have other several positive impacts. *Salix babylonica* has been found to effectively remove nitrogen and phosphorus from eutrophic water, making it a valuable wetland species (Jing and Hu, 2010). It also has potential medicinal value due to its high content of biologically active compounds (Wahab et al., 2022) with studies having identified numerous secondary metabolites in *S. babylonica*, including flavonoids, phenolic glycosides and terpenes (Tawfeek et al., 2021) which are compounds that contribute to various pharmacological activities, and have antioxidant, antimicrobial and anti-quorum sensing properties (Wahab et al., 2022). *Salix fragilis* has been shown to enhance floral diversity in cold desert areas, particularly in the Spiti valley of the North-West Himalayas by improving the micro-habitat conditions under their canopy (Rawat et al, 2009).

Salix babylonica and *Salix fragilis* in South Africa

Invasive alien *Salix* species are widespread along watercourses across the Grassland biome of South Africa (Henderson, 1991) where they were initially introduced in 1919 for a basket business in Parys, Free State (Urban and Eardley, 1995). This environment is ideal for their vegetative propagation (Poynton, 1973). Very little vegetation grows beneath their canopies, and they also take up habitat space that native riparian plants should be occupying (Henderson, 1991). Of these invasive willow species, *S. babylonica* is the most common, producing pure stands along several river stretches (Henderson, 1991) where they were initially planted to reduce gully erosion (Rowntree and Dollar, 1999). Although *S. fragilis* is less common, it is nevertheless rather noticeable in some areas of the Grassland biome (Henderson, 1991). This is a biome that holds immense ecological and economic value, making its conservation a priority. It supports rich biodiversity, including endemic and threatened species, while playing a crucial role in carbon sequestration, climate regulation, and water filtration. Grasslands sustain livelihoods through grazing, agriculture, and ecotourism, yet they face severe threats from habitat destruction, land conversion, invasive species, and climate change (De Lange and van Wilgen 2010; O'Connor and van Wilgen, 2020). Overgrazing, fire suppression, and fragmentation further endanger native species and ecosystem balance (O'Connor and van Wilgen, 2020). Given that more than half of the world's grasslands have been lost to urbanization and agriculture, conservation efforts such as protected areas, sustainable grazing, habitat restoration, and legal frameworks are critical (O'Connor and van Wilgen, 2020). Prioritizing grassland conservation ensures the preservation of biodiversity, ecosystem services, and the long-term sustainability of communities dependent on these landscapes.

The Grassland biome of South Africa is dominated by 32 invasive woody species with *S. babylonica* being the third most prominent of these species with 1069 occurrence records in the riparian zones within this biome 17 years ago (Henderson, 2007). Henderson (2007) also listed *S. fragilis* as a tree invader in this biome with a prominence value of 3.1 (with 169 occurrence records), much smaller in comparison to a prominence value of 17.3 for *S. babylonica*. Henderson's (2007) surveys also revealed that *S. babylonica* dominates most of the riparian and wetland habitats as it is the most recorded species in these areas with 1323 occurrence records (Figure 1.8) compared to only 175 records for *S. fragilis* (Figure 1.9).

Studying gender ratios of the *Salix* species is important to understand their reproductive dynamics and spread. *Salix babylonica* for instance was introduced as a horticultural commodity for basket making (Urban and Eardley, 1995) and *Salix fragilis* was introduced for erosion management (Rowntree and Dollar, 1999) and both species were likely clonally propagated. The decline in population in specifically *S. babylonica,* the once deemed invasive species, requires us to see if this species only relies on vegetative propagation in South Africa. Since *S. babylonica* is dioecious, the presence of both male and female trees is necessary for seed production. However, preliminary field observations suggest a strong female bias in populations across the study area, reducing opportunities for natural pollination and subsequent seedling recruitment.

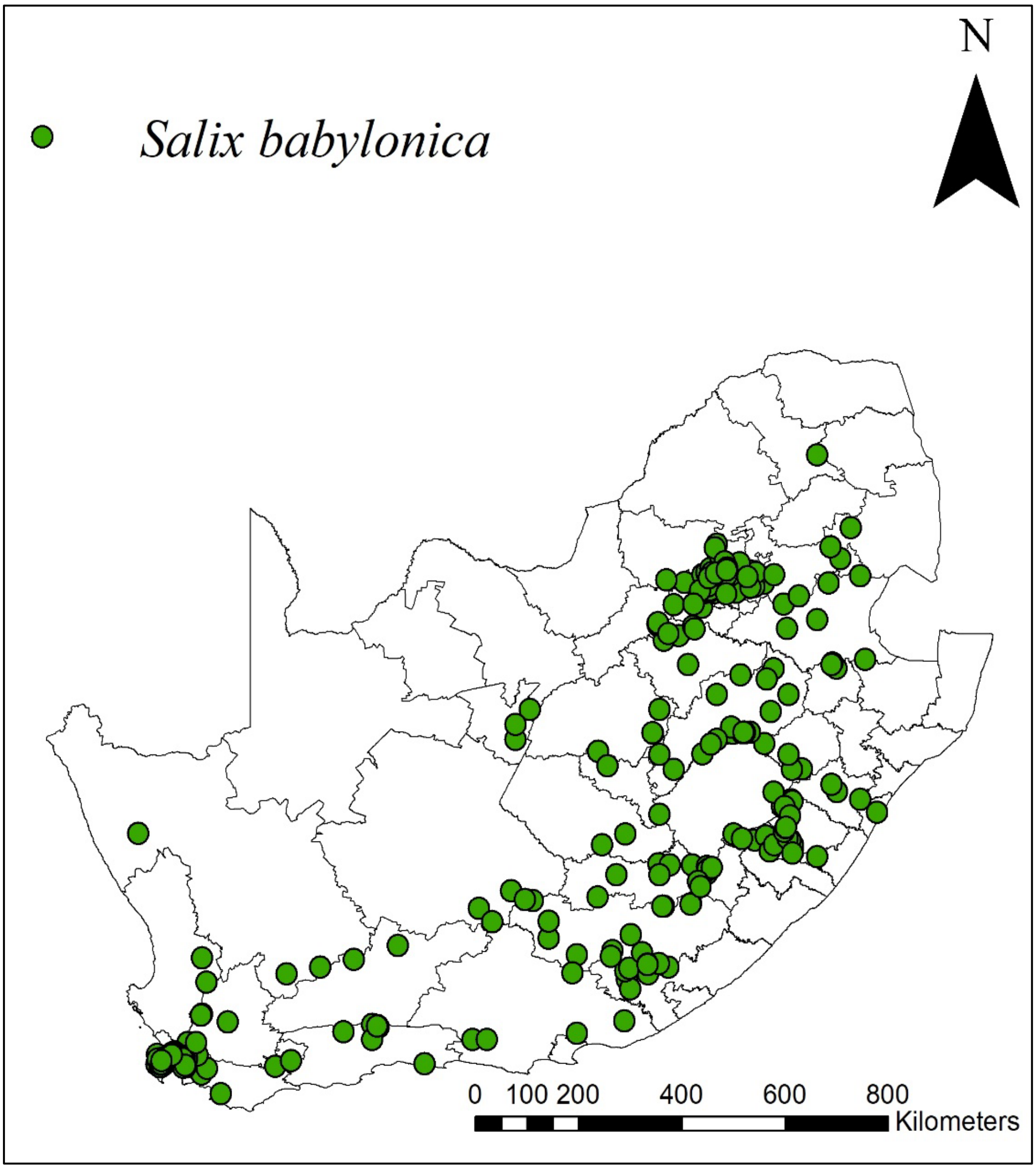

Figure 1.8: Occurrence data of *Salix babylonica* in South Africa. Green circles show recorded occurrences of *Salix babylonica*. Data are extracted from Global Biodiversity Information Facility GBIF.org (20 July 2024) GBIF Occurrence Download https://doi.org/10.15468/dl.qq7tzr.

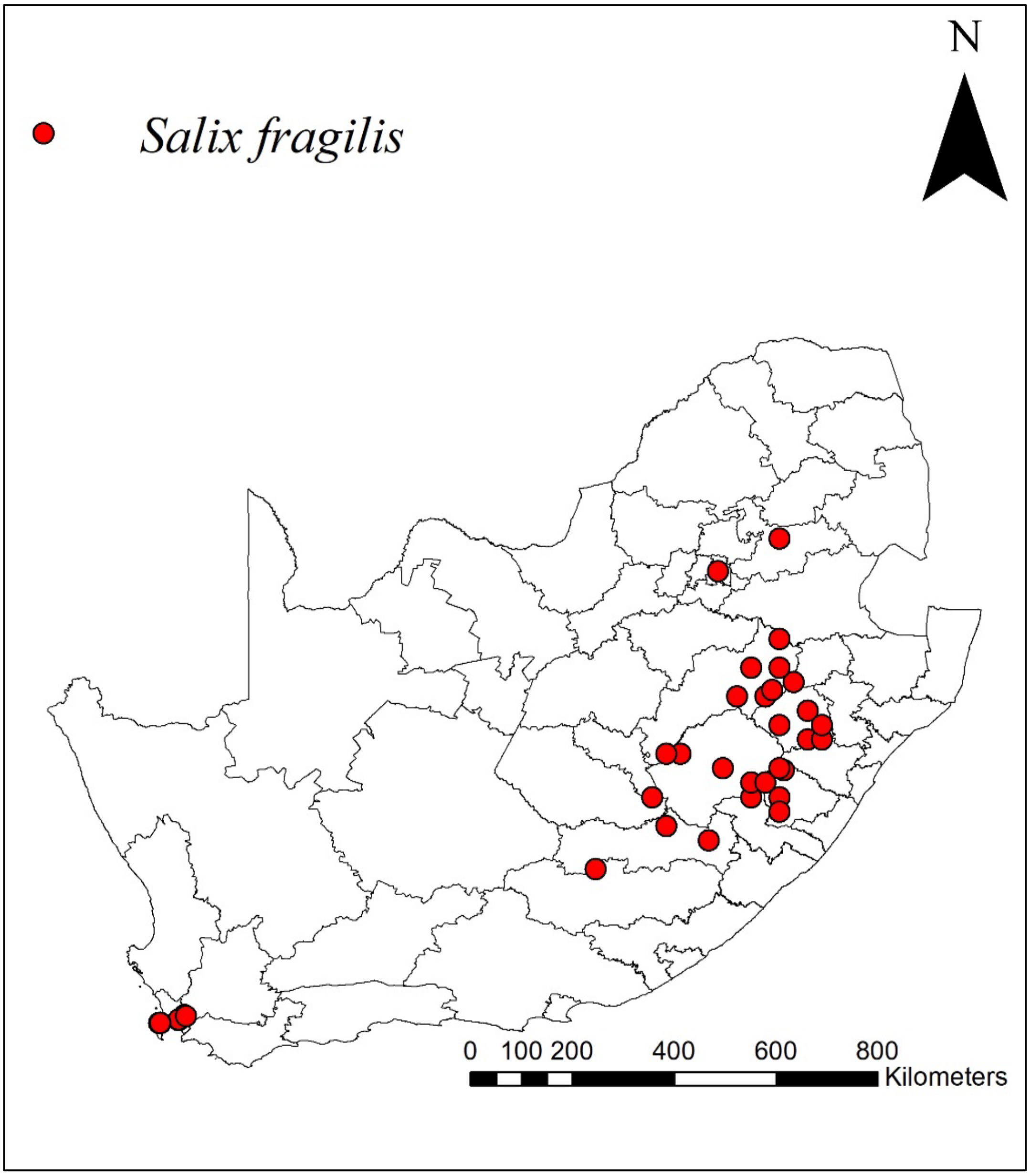

Figure 1.9: Occurrence data of *Salix fragilis* in South Africa. Red circles show recorded occurrences of *Salix fragilis*. Data extracted from Global Biodiversity Information Facility GBIF.org (20 July 2024) GBIF Occurrence Download https://doi.org/10.15468/dl.6njasa.

Legislation on *S. babylonica* and *S. fragilis* South Africa

The Conservation of Agricultural Resources Act (CARA) of 1983 (Notice 883, G. 8673), Proc. is a key piece of legislation in South Africa, focusing on soil and water conservation in agriculture (van Antwerpen et al., 2021). The purpose of this Act is to protect South Africa's natural agricultural resources by preserving the land's potential for production, preventing erosion and weakening or destruction of water sources, attacking weeds and invasive plants, and protecting the vegetation. Prior to amendments to the regulations, CARA had divided alien plants into three categories: declared weeds (Category 1 plants) and plant invaders (Category 2 and Category 3 plants). Regulation 15 of the CARA of 1983 provides a list of invasive alien plant species in South Africa, along with outlining the required management methods. Regulation 15 recognises invasive species as a danger to agricultural production and biodiversity, and the policy seeks to control and lessen their spread (CARA, 1983). According to this regulation, *S. babylonica* and *S. fragilis* are listed as Category 2 invaders. Category 2 invaders are plants that have a history of spreading and becoming invasive, but they also have certain advantageous traits that make them worth keeping around under specific conditions (CARA, 1983). These plants may be kept in demarcated areas and plantations; however, those that escape outside of these places must be managed. Neither species are listed under the 2004 National Environmental Management: Biodiversity Act (NEMBA) regulations which provide a legal framework for the management and control of invasive species in South Africa, unlike CARA that primarily deals with agricultural land and is more focused on managing invasive plants that threaten agricultural production. South Africa’s historical approach to managing invasive plants has long favoured regulation under the CAR Act (Bennett and van Sittert, 2019). Historical policy preferences, resource constraints and implementation difficulties have resulted in certain plants being regulated under the CAR Act and not being moved to NEMBA (Cronin et al., 2015; Bennett and van Sittert, 2019).

Rationale of study

Invasive species pose a significant threat to biodiversity and ecosystem functioning, and *Salix* species are known to have negative impacts on native plant and animal communities. Understanding the distribution and abundance of invasive *Salix* species in South African Grasslands can help inform management strategies for controlling their spread and mitigating their ecological impacts (Henderson, 2007). According to the last survey on *S. babylonica* and *S. fragilis* done between 1979 and 1982 by Henderson (1991) these species were regarded as highly invasive, and potentially the most problematic species in the Grassland biome. Despite this, the species have been omitted from the more recent NEMBA Act, necessitating the need for a reassessment of this species' demographics, which may allow for status updates. Furthermore, other important determinants of invasion and spread, such as reproduction and pollination ecology are overlooked in the succession of these species in new areas in previous surveys. In 2020, the Centre for Biological Control, Rhodes University, started a new programme investigating Northern Temperate weeds in South Africa (Martin, 2021). During prioritisation studies conducted on a number of invasive species the programme determined that *S. babylonica* was perceived to no longer be as troublesome as suggested in the 1980's. In addition, there were reports of limited recruitment of *S. babylonica* in some parts South Africa by landowners (G. Martin, Pers. comm.). Therefore, this study aims to determine why *S. babylonica* is still invasive in South Africa, as well as what is occurring with *S. fragilis*. It is hoped that the results from this study will provide information about the current invasive status of these species given that both *S. babylonica* and *S. fragilis* are still regarded as category 2 invasive species under the CAR Act 43 of 1983 after the last survey in 1982 (Henderson, 1991) and whether these species should be considered under the current NEMBA regulations. This study will also serve as an update on the distribution of these species, assess their population demographics to determine if they are spreading by comparing past and present survey data, and test Google Street View as a tool for virtually assessing their occurrence within the Grassland biome of South Africa.

Study aims and objectives

This study aimed to review the invasive status of *Salix babylonica* and *S. fragilis* in the Grassland biome of Eastern Free State of South Africa, assess if the population has increased or decreased over the past decades and explore the potential drivers and/or limitations to the *S. babylonica* and *S. fragilis* invasions in South Africa.

This was achieved through the following objectives:

1. Using roadside surveys and Google Street View to assess current demographics of *S. babylonica* and *S. fragilis* in the Grassland biome of Eastern Free State in South Africa and to determine whether Google Street View is an effective tool that can be used for mapping invasive tree distributions in the Grassland biome;
2. Using aerial photographs to compare past and present distributions of *S. babylonica* and *S. fragilis* in the Grassland biome in the Eastern Free State province in South Africa;
3. Assessing gender ratios of *S. babylonica* in the Grassland biome in the Eastern Free State province in South Africa; and
4. Identifying the possible natural enemies (e.g., herbivores, pathogens, or predators) associated with *Salix babylonica* and *S. fragilis* in the study area in relation to the enemy release hypothesis within the Grassland biome in the Eastern Free State province in South Africa.

Dissertation structure

The dissertation consists of five chapters: a literature review chapter, three data chapters prepared with the intention of publication in relevant peer-reviewed journals, and a conclusions and recommendations chapter.

Chapter 1: General introduction – This chapter is an overview of the history of invasive alien plant species around the globe, mainly focusing on invasive alien plants and the significance of *S. babylonica* and *S. fragilis* in the Grassland biome in South Africa.

Chapter 2: Current status of *Salix* in Grassland biome of Eastern Free State in South Africa – This first data chapter focuses on the first objective, using roadside and Google Street View to determine current distributions and population demographics of *S. babylonica* and *S. fragilis* in the Grassland biome of Eastern Free State province in South Africa. Within this chapter the gender ratio of male to female trees is checked for *S. babylonica* to ascertain if the species is capable of setting seed within the Grassland biome.

Chapter 3: Historical changes in *Salix* populations in Grassland biome of Eastern Free State in South Africa - The second data chapter focuses on comparing historical aerial images from 1980 with recent images from 2023. ArcGIS was used to collect aerial images of documented infestations of *Salix* species and assess the changes in abundance of *S. babylonica* and *S. fragilis*.

Chapter 4: Invertebrates associated with *Salix* species in the Grassland biome of Eastern Free State of South Africa - The third data chapter explores the diverse array of invertebrate species that interact with *Salix* species within the unique ecosystem of the Grassland biome in the Eastern Free State of South Africa. Through detailed observations and surveys, the chapter examines which species interact with *S. babylonica*, *S. fragilis* or both.

Chapter 5: General discussion and recommendations – This final chapter summarises the findings from all chapters and highlights the invasive potential of *S. babylonica* and *S. fragilis* as well as the accuracy of Google Street View in monitoring invasion and naturalization of alien plants. Furthermore, improvements of this study and opportunities for further research are discussed.

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CHAPTER 2

USING GOOGLE STREET VIEW TO ASSESS THE CURRENT STATUS OF *SALIX* SPECIES IN FREE STATE

Introduction

With the assistance of human migration, anthropogenic disruption of natural regions, and climate change, the rate of introduction and establishment of species outside of their native distribution has grown (Pyšek et al., 2020) affecting native biodiversity and the integrity of natural ecosystems across the world (Vilà and Hulme, 2017). The impacts cannot be left unresolved and therefore mitigation and management are required. The management of Invasive Alien Plants (IAPs) can be extremely costly and slow (van Wilgen et al., 2022). As such, any effective invasive species management strategy should include a coordinated and proactive effort to limit the import, establishment, and spread of IAPs, effectively prevention is better that cure (Randall, 1996). Preventative measures, when combined with early detection and rapid response programmes, are the most cost- and outcome-effective ways to mitigate the effects of biological invasions (Rockwell-Postel et al., 2020). The capacity to identify and effectively control new and impending invasions by alien species depends on the availability of proper survey data, which may be expensive and challenging to obtain through on-the-ground activities, particularly for larger management areas (Crall et al., 2010). Due to time and budget limitations, surveys are usually restricted to high-priority regions and frequently concentrate on a particular subset of invasive species (Baker and Bode, 2021). Efficiency and the capacity to collect reliable, relevant data must be considered when choosing survey methods (Tongco, 2007).

Invasive alien species establish more easily in disturbed areas and habitats due to less competition with native species that may have been removed or disturbed, and propagules are more likely to have dispersed to these areas during the disturbance (Mortensen et al., 2009).

Roads are disturbances that create long, narrow habitat corridors that run continuously across significant distances, which contributes to the easy dispersal of invasive alien species without being hampered by dispersal obstacles and offering opportunity for expansion into natural areas (Lelong et al., 2007, Haider et al., 2022). Within road networks, vegetation management, vehicle traffic, and road maintenance also contribute to the further spread of alien invasive plants (Gelbard and Belnap, 2003, Haider et al., 2022). These disturbances eliminate native species, thereby increasing resource availability for alien invasive plants (Mortensen et al., 2009). Given the contribution of roadsides as locations for the introduction and spread of invasive species, these regions should be prioritized when developing a surveying plan since they can be used to assess changes in invasive species' ranges as it may be easier to pick up new invasions (Shuster et al., 2005).

Since the 1970s, remote sensing techniques have been employed as a more affordable substitute for on-the-ground surveys. Over time, a variety of equipment has been accessible for these kinds of surveying techniques, from multispectral sensors to low-resolution aerial pictures (Lass et al., 2005). Digital geographic databases have grown in richness and quality over time (Parcak, 2009), and so have the methods that biologists and GIS experts may use to analyse these data to address biological demographics like those of invasive species. Among these new resources is Google Street View (GSV) application (Mazerolle and Blaney, 2011).

In May 2007, the Google Corporation introduced Street View as an addition to its Google Earth and Google Maps web mapping services (Mazerolle and Blaney, 2011). This feature allowed users to see virtual views of locations on a map from the perspective of a car travelling down a street or road (Mazerolle and Blaney, 2011). Although initially limited to a few large cities and only available in the United States in 2007 (McCarthy, 2017), Google Street View initiative has since greatly expanded its coverage. It now includes many primary and secondary rural highways and extends to regions in numerous nations worldwide. At present, there is at least partial coverage in nearly 83 countries across North America, Europe, Asia, Oceania and Africa (Mazerolle and Blaney, 2011; McCarthy, 2017; Figure 2.1). In these areas, GSV offers full-screen 360° horizontal and 290° vertical panoramic ground-level views that enable the viewer to quickly see the scene around them in any direction. Google Street View has been shown to be a valuable tool for tracking the spread of invasive species, particularly along road networks (Deus et al., 2016; Barone, 2021; Kotowska et al., 2021), providing similar results to car surveys (Barone, 2021). However, the accuracy of GSV data can vary depending on factors such as spatial scale and sampling grid size (Rousselet et al., 2013), with some studies showing decreased rates of success associated to plant conspicuity (Deus et al., 2016). Preliminary trials by Mazerolle and Blaney (2011) who investigated the use of Google Street View as a potential tool to detect, manage and monitor invasive species, suggested that taller plants such as reeds, shrubs and trees are easily detected on GSV. However, with an increase in distance from the roadside the detectability reduces (Mazerolle and Blaney, 2011). For example, the identification of *Eucalyptus globulus* Labill (Myrtaceae; Tasmanian blue gum) is difficult as trees normally appear as small individuals with a sparse distribution (Deus et al., 2016). The use of GSV is a relatively new approach to completing surveys of invasive species, and thus requires training of surveyors, as well as strict protocols for completing the surveys to ensure that accurate and reproducible data is collected. To accomplish this, one must include a data collection spreadsheet that ensures that all locations are collected for each sampling location, producing a list of random locations within the boundary of the study site to ensure sampling is unbiased, and tree identification is done by one observer (Deus et al., 2016).

Figure 2.1: Google Street View’s world coverage (dark blue) as per report from Statista (McCarthy, 2017).

One of the most serious threats to biodiversity is invasive alien plants. These alien plants tend to have a fast growth rate, long flowering and fruiting periods, higher fecundity, and more efficient seed dispersal than the native plants, giving the alien plants a competitive advantage (Rai and Singh, 2020). It is these reproductive traits that allow the alien plants to invade ecosystems, replace the native plants and alter the ecosystem completely. Among others, two invasive alien plants of interest in South Africa are *Salix babylonica* L. (Salicaceae) (weeping willow) and *S. fragilis* L. (Salicaceae) (crack willow). These species are native to China and were introduced to Africa for ornamental purposes and for lining farm roads, fields and residential areas (Henderson, 1991). These alien plants have then spread into natural areas where they became naturalized invaders, particularly along watercourses (Henderson, 1991).

The last assessment of invasive *Salix* species across the whole of South Africa was conducted in the 1980's as part of surveys to determine the distribution patterns of alien woody invasions nationally and for individual species, as well as to determine which alien woody plants are the most prominent and potentially important invaders (Henderson, 1991). Road surveys were conducted in Gauteng (Henderson and Musil, 1984), KwaZulu-Natal (Henderson, 1989) as well as Free State and north-western KwaZulu-Natal provinces (Henderson, 1991; Figure 2.2). The methodology involved continuous recordings of roadside, veld, and streambank invaders from a moving vehicle, with abundance ratings based on encounter frequency within sample units. At the end of the surveys around 1989, *S. babylonica* and *S. fragilis* were reported as the most widespread, abundant, and damaging invasive trees in the Grassland biome of South Africa, specifically with *S. babylonica* being the most abundant woody alien species in stream banks (Henderson, 1989; Henderson, 2007).

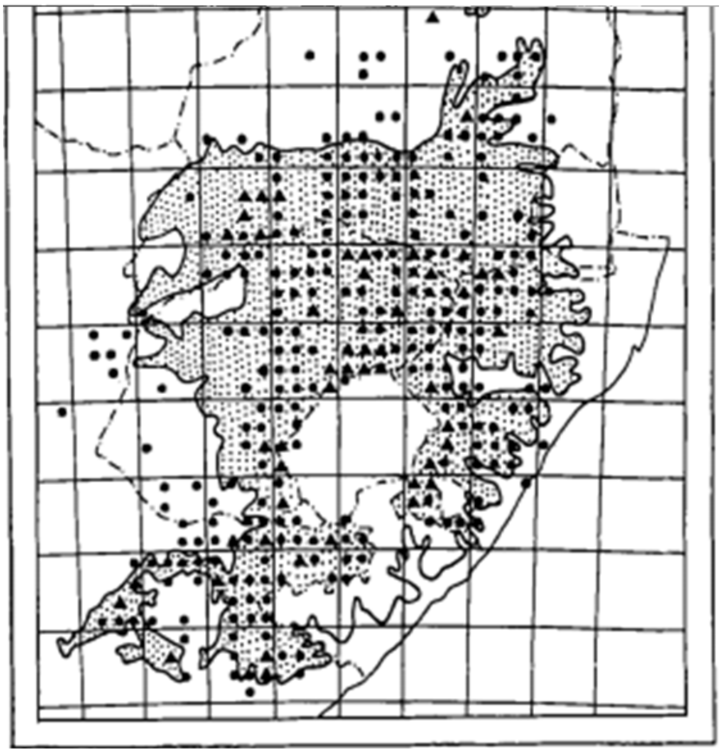

Figure 2.2: Distribution of *Salix babylonica* is the grassland biome as recorded by Henderson (1991). Triangles indicate abundances of more than five trees.

Recent observations by researchers and landowners in the Eastern Free State have suggested low recruitment (number of young trees in the environment) of *S. babylonica* suggesting the species might not be as invasive as suggested in the1980's (G. Martin, Pers. comm.). This raises the question of whether the invasive status of *S. babylonica* and *S. fragilis* has changed in the Grassland biome of South Africa since the last surveys conducted between 1979 and 1993

(Henderson, 2007). In addition, due to the observed changes in distributions of these species, there is a need to assess if these species are as invasive as reported by past literature. While alien willows were said to pose a significant ecological concern in the Grassland biome of South Africa because of their invasive nature (Henderson, 1991), there is limited understanding of the specific factors influencing the establishment, spread, and impact of alien willows in this ecosystem. It is of great importance to understand drivers of *S. babylonica* and *S. fragilis* reproduction and spread, and if they can produce viable seed. A report that there are only female *S. babylonica* plants in southern Africa except for a male plant at the University of the Free State in Bloemfontein (Jordaan, 2005) suggests that all reproduction of this species is vegetative; however, this was not confirmed through large surveys. This knowledge gap impedes the development of effective management strategies for mitigating the ecological consequences of the alien willow invasion in the Grassland biome.

It is worth noting that there are two current forms of recording invasive species distributions in South Africa. The first one is, the South African Plant Invaders Atlas (SAPIA) has been the premier source of data on the distribution of IAPs in South Africa (Henderson, 2007). SAPIA was established to collate data on the distribution, abundance and habitat types of IAPs growing outside of cultivation in southern Africa. However, the database has some limitations in sampling intensity and coverage as the records have been collected opportunistically and are restricted to roadsides (Henderson and Wilson, 2017), and records are also limited to the knowledge of those who submit records, who tend to record species that are more recognizable as opposed to those that may be more obscure. Moreover, no new records have been added since 2018. Despite these limitations, SAPIA suggests a broad invasion of *Salix* species in South Africa.

The second dominant platform for capturing invasive species occurrence records is through the online platform iNaturalist (www.inaturalist.org), which has been increasingly used to monitor

IAPs distribution. As of 28 November 2024, there were 13 177 verifiable research-grade records on iNaturalist of *Salix* species in South Africa (iNaturalist, 2024a, 2024b).

This chapter aims to review the population demographics—including gender ratios, age structure, and distribution—of *Salix babylonica* and *Salix fragilis* in the Grassland biome of the eastern Free State, South Africa. To achieve this, data on the distribution of *Salix* species was first collected remotely using Google Street View, which was then verified in situ through ground-truthing via traditional in-person road surveys to determine if Google Street View is a feasible method to map *Salix* species distributions in the Grassland biome. In addition, road surveys were conducted to assess the age demographics of *Salix* populations. During the road surveys the gender ratio of male to female trees is checked for *S. babylonica* to ascertain if the species is capable of setting seed within the Grassland biome.

Material and Methods

Study species

The weeping willow (*Salix babylonica* L., Salicaceae) is a deciduous tree native to the arid regions of Northern China. It is also found in other parts of Asia, where it has been cultivated and later traded to Europe and America (Bailey, 1949). The species has been introduced globally, including southern Africa, where it is often considered invasive (Henderson, 1991). This broad-headed tree is characterized by slender branchlets that hang vertically, nearly reaching the ground, and can grow up to 25 meters tall at maturity (Henderson, 2007). Its drooping branches form dense, tall canopies—up to 6 meters wide—commonly found along riversides and dam edges. The weeping willow grows rapidly, thrives in wetland environments, and can reach altitudes of up to 1600 meters (Popova and Kaleva, 2015). Its vegetative propagation allows it to invade riverbanks and agricultural margins in warm regions worldwide (McInerney et al., 2021). This clonal species is primarily spread through human activities and river flooding (Henderson, 1991; Kennedy et al., 2003). It is commonly found along roads and waterways in regions like South Africa and Australia (Henderson, 1991; Kennedy et al., 2003; Mararakanye et al., 2017). Adapted to warmer climates, the weeping willow is sensitive to cold temperatures (Malone and Ashworth, 1991) and depends on abundant water during its growing season, which explains its preference for riparian habitats (Henderson, 1991).

The crack willow (*Salix fragilis* L., Salicaceae) is a medium to large deciduous tree that grows rapidly, reaching heights of up to 15 meters and a trunk diameter of up to 1 meter (Henderson, 2007). It often has multiple trunks, with smooth branchlets that ascend irregularly, forming a crown-like shape (Henderson, 2007; Dirr, 2009). The bark is dark grey-brown and becomes coarsely fissured with age. Its lance-shaped leaves are bright green, with finely serrated edges, and are initially covered with fine hairs in spring, but quickly become smooth and hairless. The tree produces dioecious flowers in the form of catkins (male and female catkins occur on separate trees) during early spring (Henderson, 2007). By late spring, its fruit capsules release numerous small, cotton-tufted seeds that are easily dispersed by wind and water, germinating immediately upon making contact with soil (Henderson, 2007).

Sampling Methods

Google Street View survey

Ten populations of *Salix* trees were pre-surveyed in person for the observer to familiarize themselves with what the trees looked like from the road and when "driving" using GSV. Between December 2022 and January 2023, a comprehensive, virtual tree survey was conducted in GSV using a ground-level, side-view perspective of the streetscape to compare with a ground-level field survey to locate and survey willow populations located along or nearby the main roads within the Grassland biome in the Eastern Free State. Observation of *S. babylonica* and *S. fragilis* populations surveyed using GSV virtual images was conducted along ~ 500km of road network within the grassland biome specifically along the N5 road (Phuthaditjhaba to Bloemfontein), located between Maluti-a-Phofung Local Municipality and

Mangaung Municipality (between 28°33′04″S 29°04′48″E and 29°6'46.44" S 26° 12'53.64" E and the R712 road (between Harrismith and Clarens) stretching between the Maluti-a-Phofung Local Municipality and Dihlabeng Municipality (between coordinates 28°33′04″S 29°04′48″E and 28°21'41.4"S 28°17'42.4"E) and the N3 (Harrismith to Johannesburg). A total of 766 occurrence were recorded.

Following the virtual survey, 30 occurrence records were randomly selected from the location database of locations and visited for ground-truthing within a month of finishing the GSV survey. The GSV virtual survey tool was accessed using the freely available images in Google Earth Pro (version 7.3) with images that ranged from as early as 2010 and to more recent images taken in 2023 (Figure 2.3).

Figure 2.3: Examples of roadside images of *Salix babylonica* in Google Street View in captured by the Google Street View Car in (A) 2022 and (B) 2023.

Road survey

A road survey was performed to determine the population demography and gender ratio of *S. babylonica* and *S. fragilis* in the Grassland biome. Roadsides were defined as strips of public or private land on either side of the roads but were limited to 100 metres perpendicular to the road. Road surveys were performed between July 2022 and January 2023 by three observers. A total of six routes were taken along the main roads within the Grassland Biome, namely the N3, N5, N6, R712 and R57, some of which were routes also surveyed in the Google Street Survey. Each route was selected according to the following criteria: Each route should a) not have been travelled by the observer before during the road side survey; b) be a main national or regional road within the Grassland biome; and c) be able to be driven in both directions so that the observer could safely assess populations on both sides of the road unobscured by the road's width (Deus et al., 2016). For each population, the species of willow (*S. babylonica* and/or *S. fragilis*) was recorded, and tree abundance was estimated using a scale (1-5; 6-20; >20 trees). To make the data comparable to the GSV survey, the age of trees closest to the observer (i.e. representing the GSV Car) was estimated according to Table 2.1 with visual examples in Figure 2.4. If catkins were visible, gender was recorded. The observer got out the car and examined the gender of each trees they could get close to. Female trees produce catkins with only pistils which differentiates from the male trees whose catkins have stamens only (Henderson, 2020).A total of 366 roadside sites were assessed along ca. 1694 km. Site locations were recorded using a GPS application (Find My GPS Coordinates, Youngmin Koo, version 2.0.63, developed in 2012).

Table 2.1: Scale used to assess the age of the *Salix* trees

Tree Age	Description
1	Seedling < 2 m
2	Young tree >2 m; <30 cm at breast height

3	Mature tree (healthy with no broken branches, trunk intact)
4	Mature tree (one or two broken branches, trunk intact)
5	Mature tree (many broken branches, trunk intact)
6	Mature tree (many broken branches, trunk broken)
7	Mature tree (only broken trunk remains, one or two shoots remain)
8	Mature tree (completely dead or rotting trunk remains)

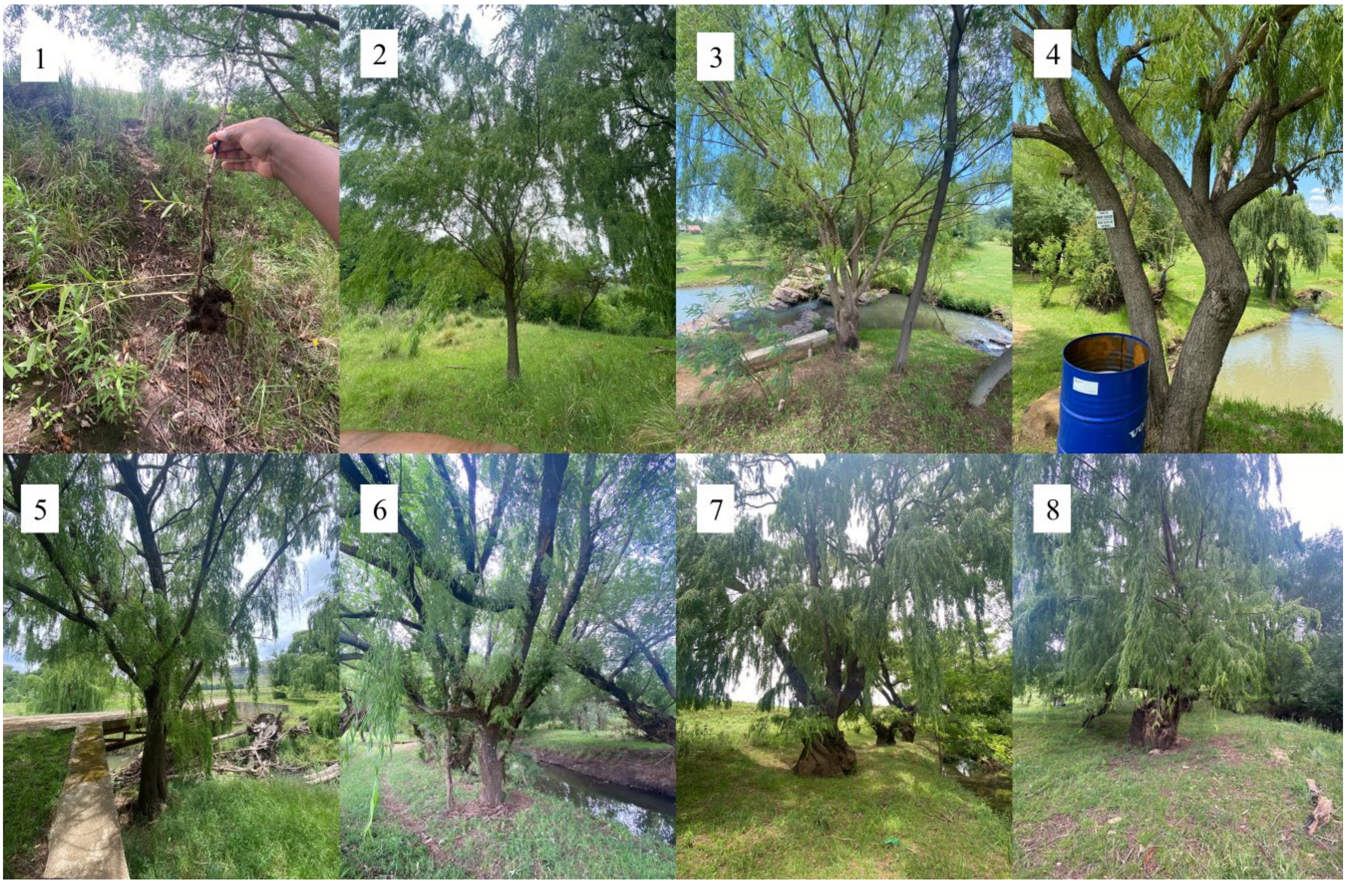

Figure 2.4. Images of each age classification used in Table 2.1 Showing age 1-seedling up to age 8- Mature tree with old trunk/dead trunk. Photo credit: Mashamba T.

Data analysis

Google Street View Survey

The data points collected on Google Street view were downloaded as Keyhole Markup Language (KML) files that were analysed in Microsoft Excel. The data was converted to comma-separated values (CSV) format to create spatial maps showing the distribution of *S. babylonica* and *S. fragilis* using Arc Map version 10.7.1.

Roadside survey

The data points collected were manually entered in Microsoft Excel, recording the relevant information accompanying each point, i.e. estimated tree age and population size at each site. This information was used to create graphs showing how many trees were of each age class. These data were converted to CSV format in order to create spatial maps showing the distribution of *S. babylonica* and *S. fragilis* by age in Arc Map version 10.7.1.

Results

Google Street View

A total road distance of 417 km was surveyed during the GSV virtual survey. Trees were recorded on either side of these roads, growing in clusters of either *S. babylonica*, *S. fragilis* or a mix of the two species (Figure 2.5). Some willow populations identified through GSV contained both *S. babylonica* and *S. fragilis* trees, but *S. babylonica* was the most frequent willow species observed in these mixed stands. Pure *S. babylonica* stands made up 82 % of points surveyed (Figure 2.6), 7 % of the stands were pure stands of *S. fragilis* and 11 % of the stands observed contained both *S. fragilis* and *S. babylonica*. Both *Salix* species occurred along riverbanks and other water bodies (e.g. dams), roadside picnic spots or open veldt. *Salix fragilis* was more difficult to observe on GSV, and accurate observations could only be made when trees were growing along riverbanks and when images were taken while the trees had leaves. When detected, *S. fragilis* was mostly found as continuous, almost pure, stands on riverbanks or other water sources like dams and not as individual trees. Pure stands could stretch for many kilometres, such as along the Little Caledon River near Clarens in Free State (Figure 2.7). Of the 765 points observed on GSV, 30 points were visited for ground truthing and both *S. babylonica* and *S. fragilis* were correctly identified via GSV at all 30 sites.

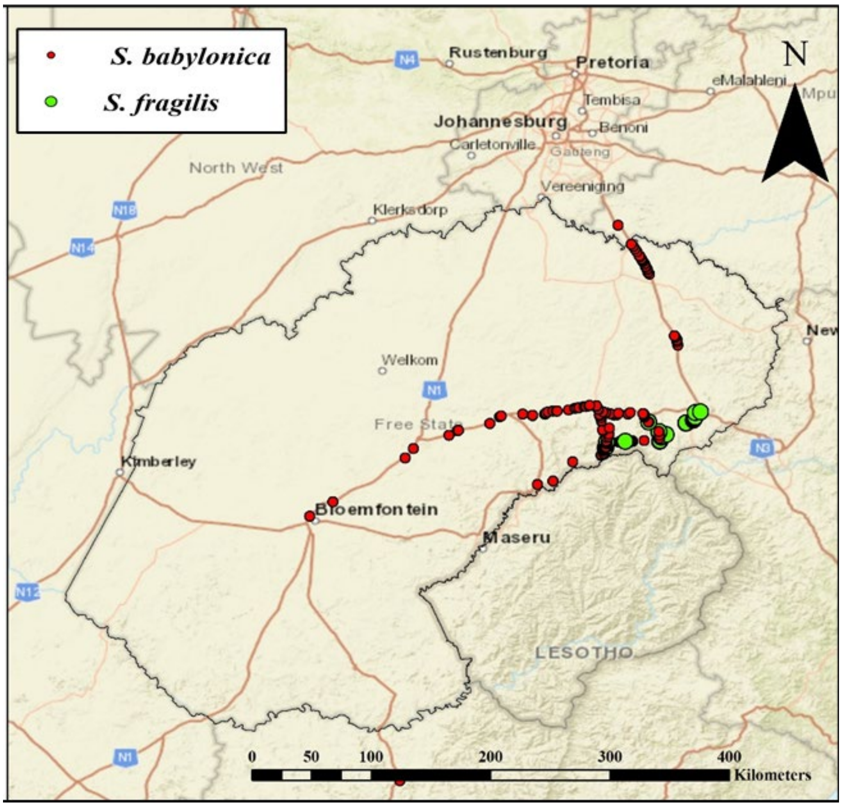

Figure 2.5: Distribution of *Salix babylonica* (red dots) and *Salix fragilis* (green dots) identified in a Google Street View survey withing the Grassland biome in the Free State Province of South Africa.

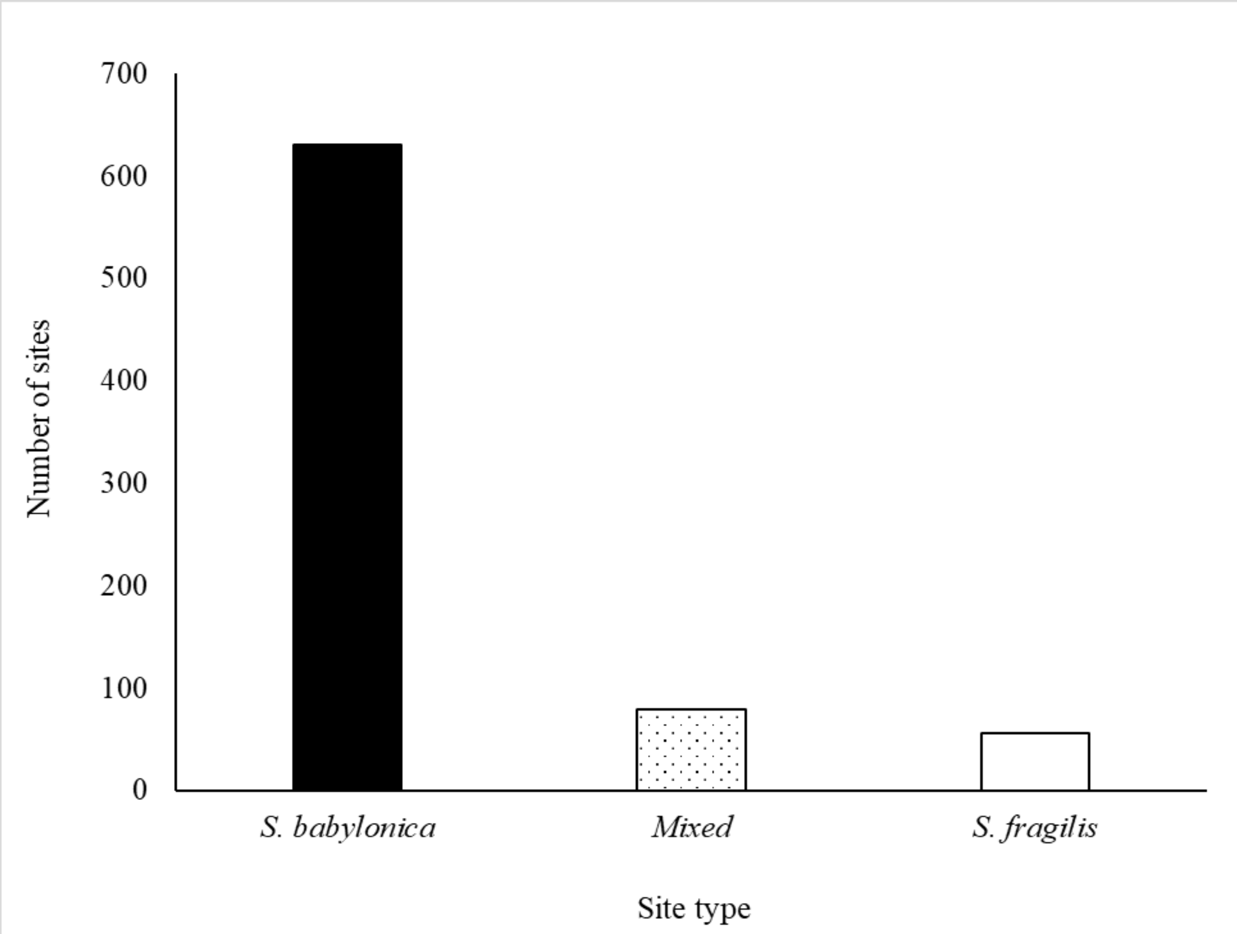

Figure 2.6: Comparison of number of sites from GSV surveys containing pure *Salix babylonica* stands, pure *S. fragilis* stands and mixed stands along watercourses and roadsides in the Grassland biome in the Free State Province of South Africa.

Figure 2.7: A pure stand of *Salix fragilis* identified in a (A) road survey and (B) as seen on Google Street View, along the Little Caledon River in the Free State Province of South Africa.

Roadside Survey

Salix babylonica is more widely distributed than *S. fragilis* (Figure 2.8). Most of the *S. babylonica* stands were made up of individual trees along the roadside with approximately 50% of the sites having a population size of 1-5 trees (Figure 2.9). There appears to be extremely low levels of recruitment for *S. babylonica* with 78% of the trees being recorded as mature old trees showing signs of old age (e.g. broken main branches; Age 5-8) with 99.3% of all the trees surveyed trees being recorded as mature (Age 3-8) (Figure 2.10 and Figure 2.11).

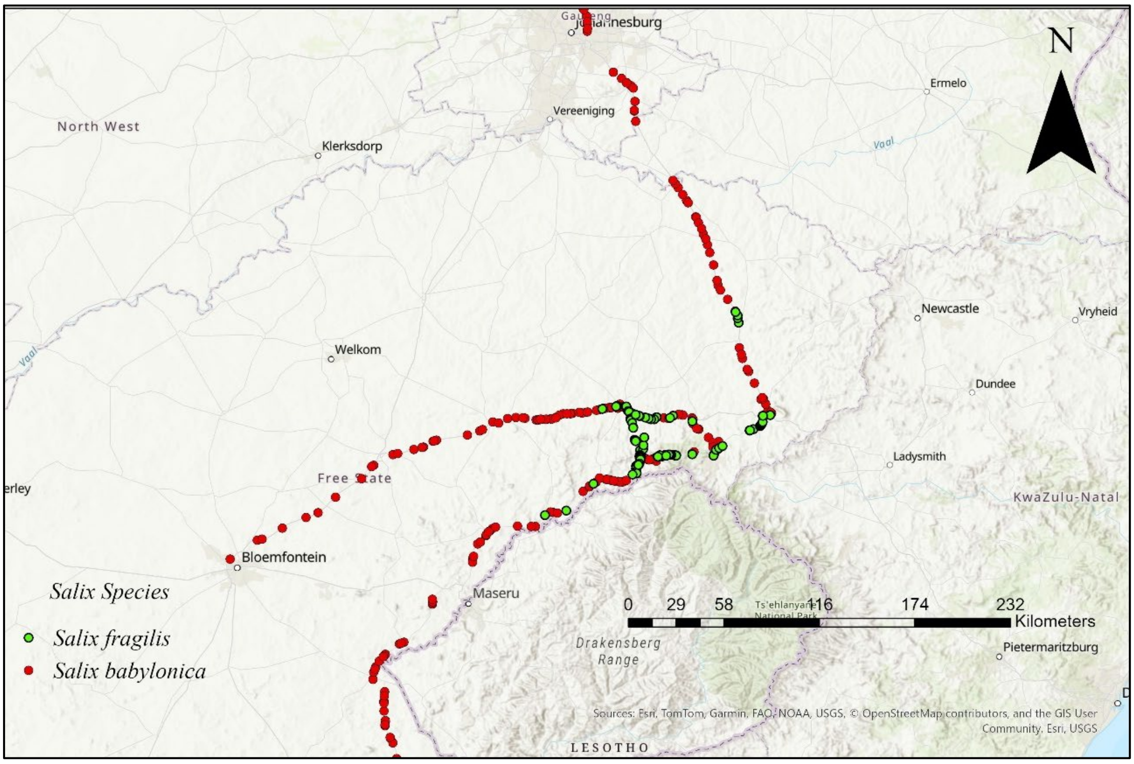

Figure 2.8: Distribution of *Salix babylonica* (red dots) and *Salix fragilis* (green dots) along the roads surveyed in within the Grassland Biome of South Africa.

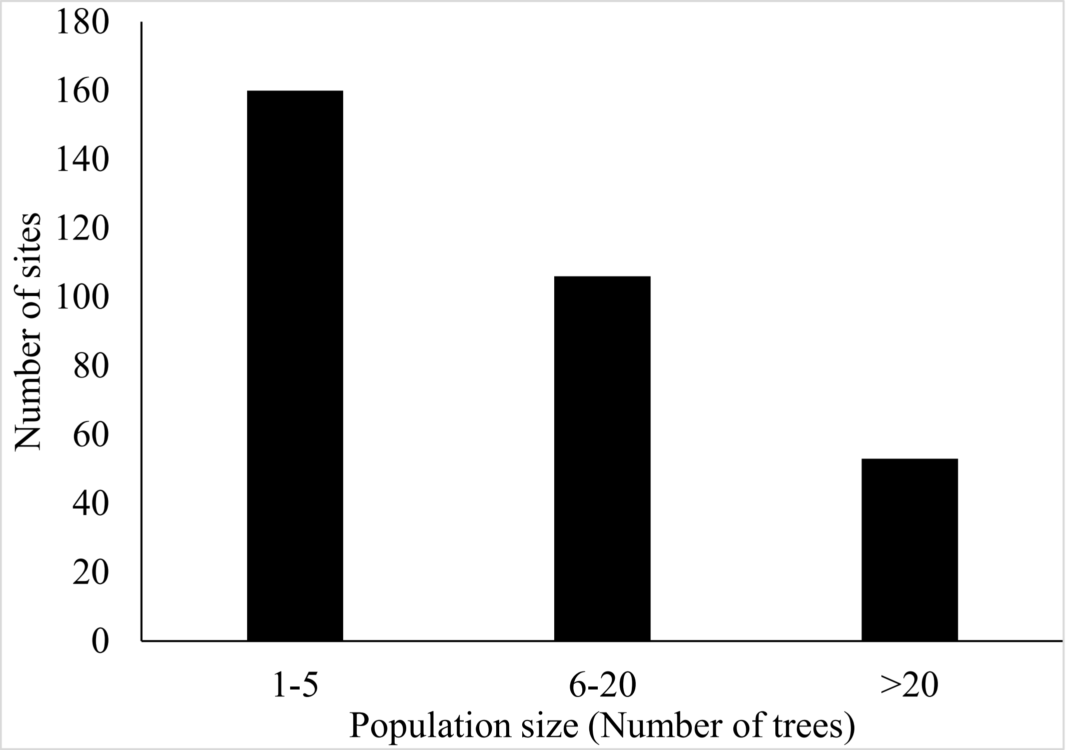

Figure 2.9: Average population sizes of *Salix babylonica* trees recorded during a Road survey along main roads within the Grassland Biome, namely the N3, N5, N6, R712 and R57.

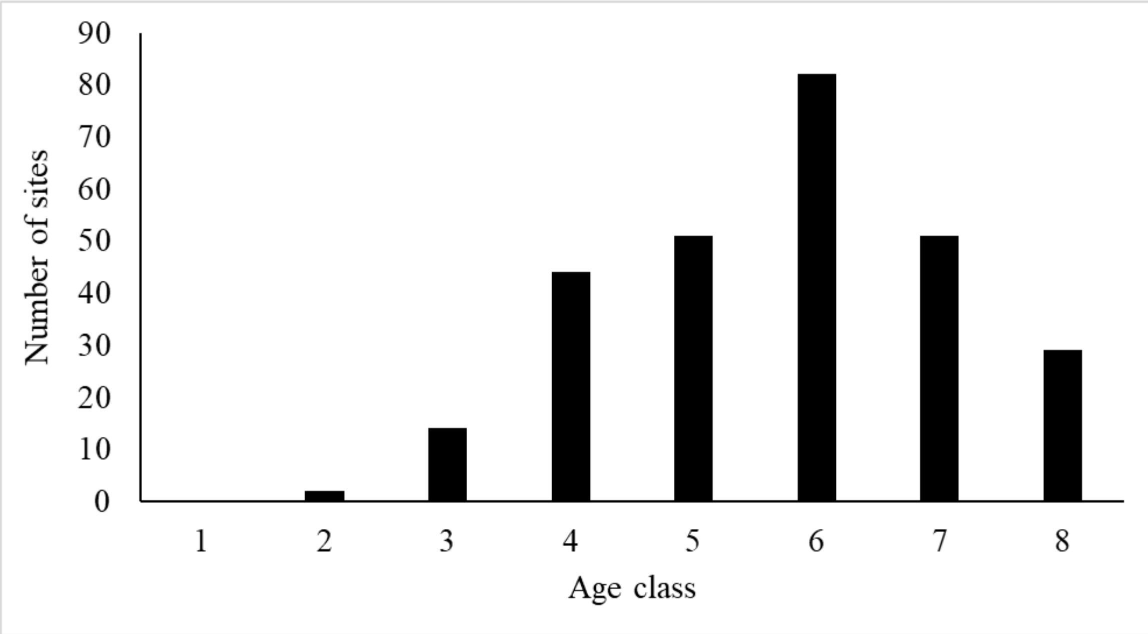

Figure 2.10: Number of sites for each age class of *Salix babylonica* recorded during a Road survey along main roads within the Grassland Biome, namely the N3, N5, N6, R712 and R57.

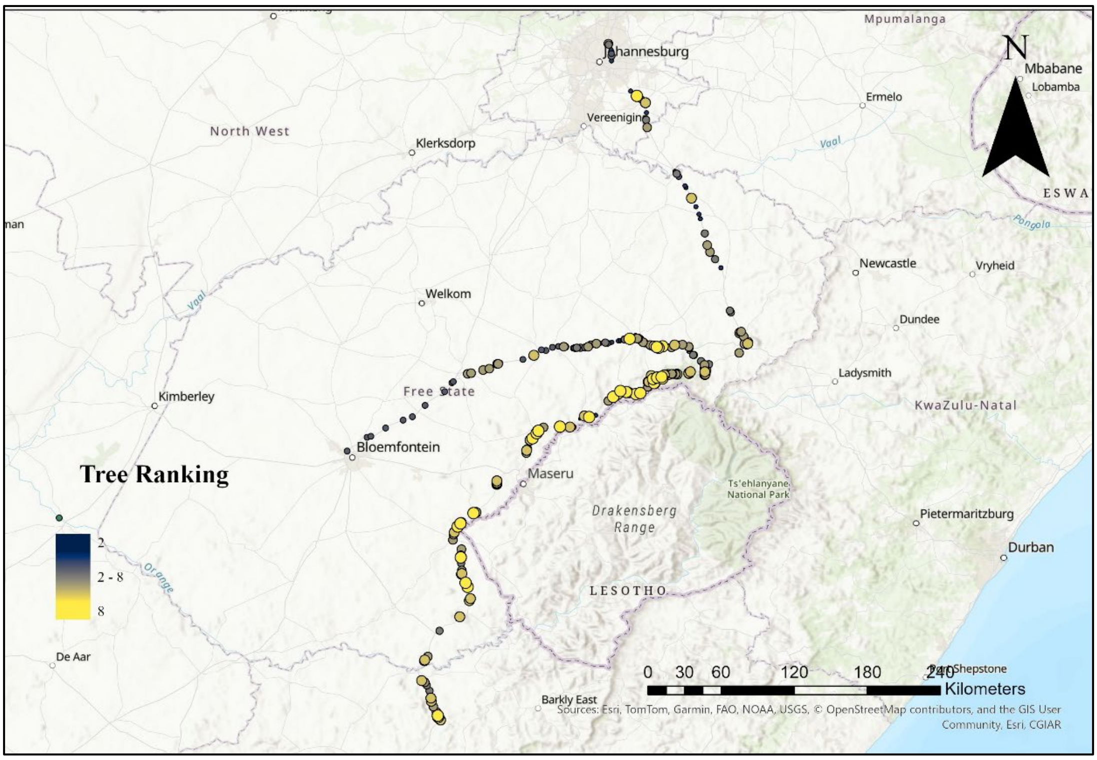

Figure 2.11: Age Distribution of *Salix babylonica* along the roads surveyed in within the Grassland Biome of South Africa ranking age 1-seedling up to age 8- Mature tree with old trunk/dead trunk.

Recorded *S. fragilis* trees were primarily aged between rankings 3 and 7, with 36% having an age ranking of 6 (mature tree (Many broken branches, trunk broken)), followed by trees with the age ranking of 3 (mature tree) with 29%. Recruitment was observed for *S. fragilis* with 44% of the trees recorded as seedling, young tree, or mature tree (Age 1-3) (Figure 2.12). The distribution of *S. fragilis* is more localized in the eastern parts of Free State (Figure 2.13). Compared to *S. babylonica*, *S. fragilis* sites appeared to have more trees, with 56% of the sites having 6 or more trees (Figure 2.14).

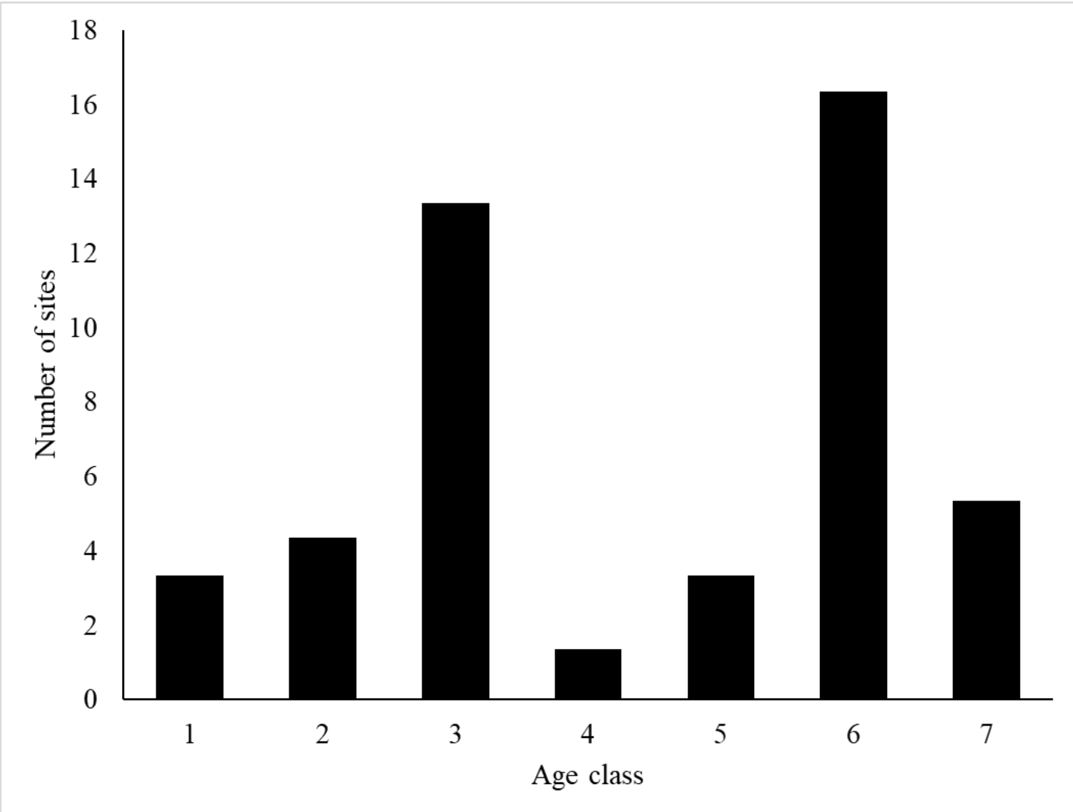

Figure 2.12: Number of sites for each age class of *Salix fragilis* recorded during a Road survey along main roads within the Grassland Biome, namely the N3, N5, N6, R712 and R57.

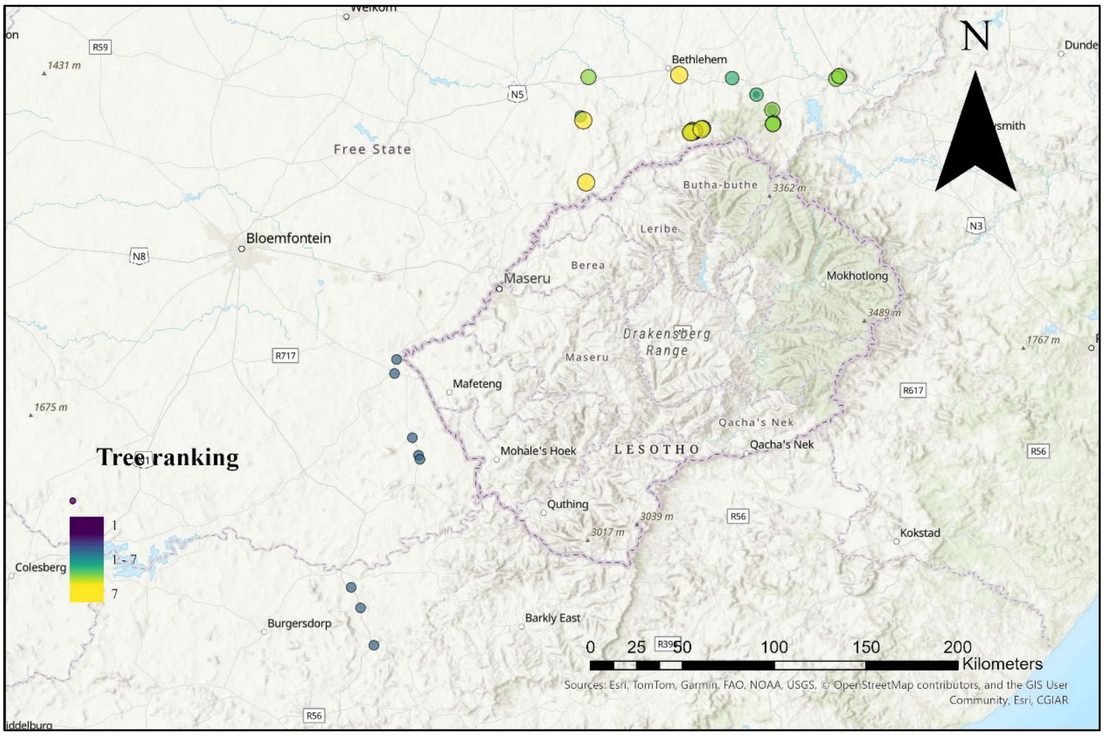

Figure 2.13: Age Distribution of *Salix fragilis* along the roads surveyed in within the Grassland Biome of South Africa ranking age 1-seedling up to age 8- Mature tree with old trunk/dead trunk.

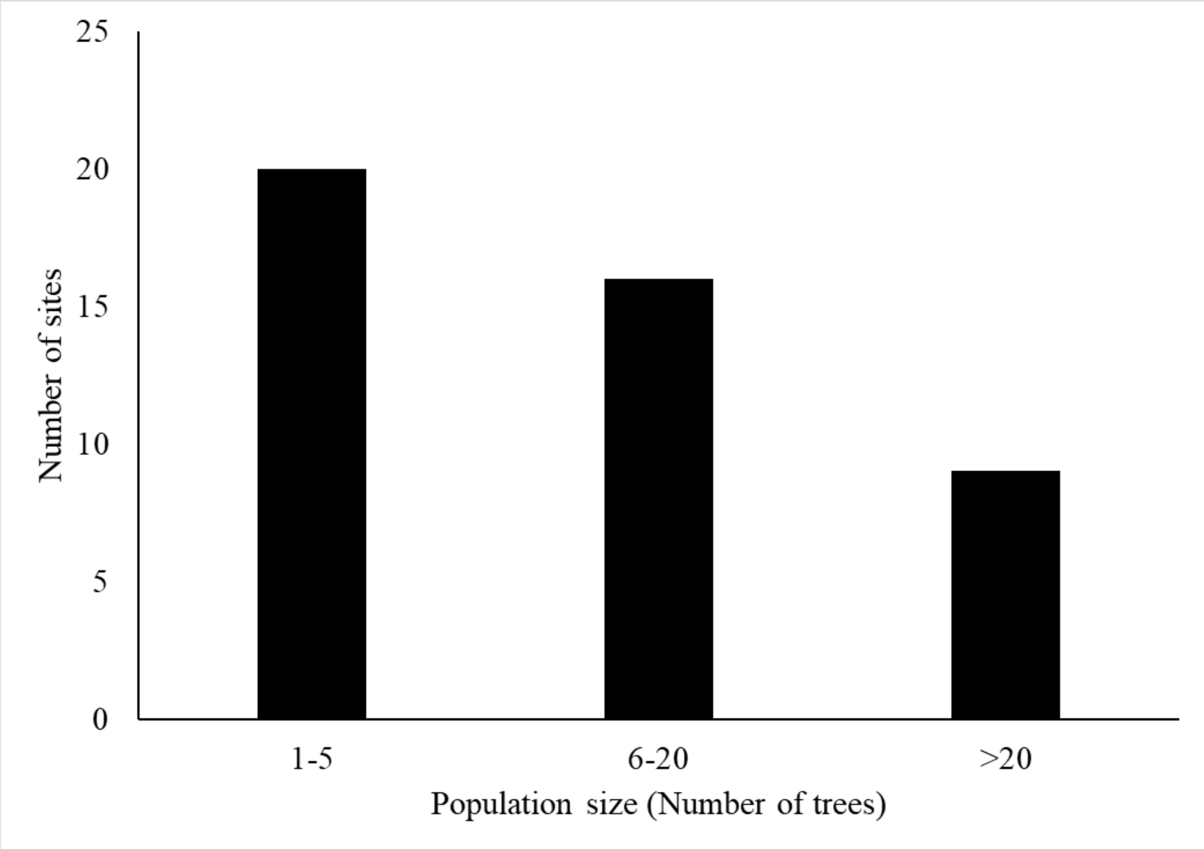

Figure 2.14: Average population size per number site of *Salix fragilis* trees recorded during road surveys along main roads within the Grassland Biome, namely the N3, N5, N6, R712 and R57.

All *Salix babylonica* trees surveyed along the road were identified as female trees. No male tree(s) could not be located, and this suggests there are no male trees in South Africa.

Discussion

Google street view surveys

Google Street View was shown to be a useful tool for mapping the distribution of both *Salix* species studied, with identification confirmed through ground-truthing. This was a rather positive outcome compared to its use for tracking tree health and population numbers for other tree species such as *Eucalyptus globulus* Labill (Myrtaceae) also known as the Tasmanian blue gum (Deus et al., 2016), as both *S. babylonica* and *S. fragilis* are bigger individuals, which are easier to recognise, than *Eucalyptus globulus*.

It was challenging to identify *S. fragilis* from the imagery taken in winter due to a more traditional upright shape whereas as *S. babylonica* was easily identified due to its characteristic shape, even in winter (Figure 2.15). A major limitation of GSV imagery is the lack of control of season and date at which the image was taken, especially when trying to make comparisons between sets of imagery taken at different times.

Salix babylonica was more abundant over the survey areas, whereas *S. fragilis* was found in less locations; however, *S. fragilis* was usually found in larger populations, sometimes forming monocultures along stream and riverbanks. *Salix babylonica* was easily identified and seen often as a stand-alone tree, probably due to being planted by landowners. A few localities had mixed stands of both species, often also with other tree species such as *Populus* × *canescens* (Aiton) Sm (Salicaceae). *Salix fragilis* populations were always growing in river or dam systems, whereas *S. babylonica* was observed to grow in open grassland on occasion.

Figure 2.15: A mixed stand of *Salix fragilis* (in red circles) and *Salix babylonica* after winter along the R74 in the Nuwejaarspruit river in the Free State province in South Africa.

Roadside surveys Results

Very few juvenile trees were observed for *S. babylonica* whereas a larger proportion of *S. fragilis* populations were classified in younger age classes. For *S. babylonica*, 78% of trees were classified in age groups 5 (mature tree with visible aging) to 8 (broken trunk to senesced). For *S. fragilis*, 44% were observed between age groups 1 (sapling) to 3 (mature healthy tree). *Salix babylonica* and *S. fragilis* can propagate both sexually and vegetatively (Henderson, 1991). Our surveys suggest that only female trees of these species are available in South Africa. The large populations of young *S. fragilis* trees compared to *S. babylonica*, suggest that *S. fragilis* is successfully propagating vegetatively given that both species only have female trees in their populations. The minimum and maximum mean annual temperature in South Africa, has increased at least 1.5 times greater than the observed global average increase of 1-2°C during the last 50 years according to the Long-Term Adaptation Scenarios (LTAS). Aridity in southern Africa increased by 10.8% during the period 1980–2007, exposing South Africa to water scarcity (Nhemachena et al., 2020). In other species under the Salicaceae family, such as *Populus* hybrids, certain environmental conditions during propagation (e.g. temperature and water availability) can affect rooting during vegetative propagation (Gudynaitė-Franckevičien and Pliūra, 2021). Similarly, the reduction in water in the Grassland biome could be directly affecting the successful vegetative propagation of *S. babylonica* because increased water content in most plants enhances regeneration capacity (Yıldız et al., 2016).

The GSV surveys showed that most *S. babylonica* trees were stand-alone whereas more than 55% of the populations of *S. fragilis* consisted of more than six individuals. *Salix babylonica* was found throughout the Free State and extending into Eastern Cape, KwaZulu-Natal, Gauteng, and Limpopo, whereas *S. fragilis* was only observed in the eastern Free State. This could be because *S. babylonica* is actively planted as an ornamental plant resulting in a wider distribution. *Salix fragilis* might not have any human involvement facilitating its propagation outside of the areas it is already established in.

One of the main challenges faced during the GSV surveys was the seasonal variability in the visibility of *Salix* species, as they lose their leaves in winter. One would usually consider conducting surveys during peak growing seasons to enhance the visibility of both species; however, this cannot be altered as the Google Street vehicle does random updates of the panoramic images. Future research should compare the effectiveness of GSV and traditional roadside surveys across different invasive species. This could include analyzing species that exhibit different growth forms or habitat preferences, providing insight into which method yields more reliable data for specific contexts.

Both GSV and roadside surveys offer valuable insights, but they serve different purposes. Google Street View is a cost-effective method for large-scale surveys, allowing for rapid data collection over extensive areas for *S. babylonica* and *S. fragilis*. It is especially beneficial for preliminary surveys and when accessibility is a concern. Conversely, roadside surveys provide detailed information about tree health, age demographics, and site-specific conditions that GSV may not capture. This detail is essential for understanding local ecological contexts and species interactions. These two methods can be seen as complementary rather than directly comparable methods. Each has its strengths and weaknesses and using them in tandem could yield more comprehensive insights into the distribution and health of *Salix* species and other invasive plants.

The historical literature on *Salix* species in South Africa suggests that *S. babylonica* is the most widespread riverine invader in the grasslands (Henderson, 2007), forming continuous stands along major perennial watercourses due to its self-propagation and floodwater dispersal. *Salix fragilis* is similarly propagated and has been known to form pure stands along entire river reaches (Henderson, 1991). Both species pose a significant threat to the conservation of indigenous riparian flora and alter the hydrology of the invaded watercourses. However, contrary to these historical findings, this research indicates a potential shift in dominance between the two species. While *S. babylonica* appears to be in decline, *S. fragilis* may be emerging as a more significant invasive species. This suggests that the ecological dynamics of these species in South Africa's riparian zones may be changing.

Conclusions

Google Street View is a reliable tool to identify *S. babylonica* and *S. fragilis* along roadsides in South Africa with the reliability diminishing for *S. fragilis* if GSV photos were taken in winter season, when the trees lose their leaves. Both Google Street View and roadside surveys confirm that *S. babylonica* is more widely distributed than *S. fragilis*. However, wherever *S. fragilis* is located it is in higher density than *S. babylonica*. Only female *S. babylonica* plants were recorded in South Africa. There is no recruitment of *S. babylonica* and the population is made up of mature and dead trees. Although *S. fragilis* is not as widely distributed there is recruitment to its population and potential to increase in its distribution compared to *S. babylonica*.

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CHAPTER 3

HISTORICAL CHANGES IN *SALIX* POPULATIONS IN THE EASTERN FREE STATE PROVINCE, SOUTH AFRICA

Introduction

Invasive alien plant (IAP) species in South Africa have increased significantly over time, with the number of taxa regarded as invasive rising from 548 in 2000 to 773 in 2016 (Henderson, 2007; Henderson and Wilson, 2017) with 8700 individual records having been recorded in SAPIA (van Wilgen, 2020). The geographical extent of these invasions has also expanded, with a 50% increase in quarter-degree squares occupied by IAPs between 2000 and 2016 (Henderson and Wilson, 2017). The Forest, Fynbos, Grassland, and Savanna biomes are particularly affected by alien tree and shrub invasions, with *Acacia* species being the most prominent invaders in these ecosystems (Henderson, 2007). Invasions by alien trees and shrubs have substantial impacts on ecosystems, including native species loss, increased biomass, and altered fire regimes (Nel et al., 2004; Chamier et al., 2012). Water resources are significantly affected, with invasive trees such as *Salix fragilis* and *Salix babylonica* reducing stream flow and altering nutrient cycles, thereby impacting water quantity and quality (Henderson, 1991: Chamier et al., 2012). Management efforts, including biological control, have shown success on a limited number of species in reducing the spread of certain species, but more strategic interventions are needed to address this growing problem (Nel et al., 2004; Henderson and Wilson, 2017).

Invasive alien plants have unique characteristics that enable them to spread rapidly and cover vast territories in locations where they are not native (Jeschke et al., 2012). A primary question in invasion biology is: what will the rate of spread of an alien invader be after the initial establishment (Hastings, 1996)? The issue of invasion dynamics also has a practical aspect. The rate of spread has been long recognized as one of the parameters that enables managers to know if an alien invasive species needs to be controlled, as alien species with high rates of spread are likely to become more widely distributed and troublesome (Forcella, 1985). Methods used to assess the dynamics of invasion in the past vary with the aims of the study and each provide different information (Hulme, 2003). For example, at regional to continental scales, herbarium records are informative (Delisle et al., 2003) as these provide information on the plants that have been seen and collected in an area over time, but such data are usually not informative regarding the increase of area covered by the invader over time or of any of the impacts they may be having.

Another method for assessing the change in invasions over time involves the use of remote sensing for monitoring invasive species (Müllerová et al., 2005). Aerial photographs have been used as a tool for quantitative assessment of alien plant infestations, most often obtained using remote-sensing techniques, by detecting plant species, providing area estimates of alien plant populations (Higgins and Richardson, 1996; Everitt, 1998; McCormick, 1999; Stow et al., 2000) and giving information on the dynamics of their spread (Mast et al., 1997). Aerial photographs can be used to analyse the dynamics and extent of invasion on local scales, making it possible to answer important questions in invasion biology, such as the spatial extents and dynamics of the invasions by an alien species and how some parameters of the species' population dynamics change over time (Tiley et al., 1996). Improved data collection and sharing can enhance the ability to track progression of invasions of any kind, identify emerging threats, and assess current and future risks (Vanderhoeven et al., 2017). Moreover, monitoring the spread of these IAPs is vital for developing and implementing effective control measures (Shackleton et al., 2020).

Aerial photographs in tree population assessments

Aerial photography has long been a valuable tool in environmental science, providing a unique perspective for assessing and monitoring changes in landscapes over time (Morgan et al., 2010). Aerial photography has been widely used in tree population assessments due to its ability to capture the vertical dimension of vegetation and assess changes over time (Fensham et al., 2002). More recent studies have explored the use of Unmanned Aerial Vehicle aerial photographs for population estimation, with promising results (Permana and Saputri, 2022).

Several studies have used aerial photography to map changes in populations of IAPs. For example, aerial photography was used to map three riparian invasive species *Impatiens glandulifera* Royle (Balsaminaceae)*, Heracleum mantegazzianum* (Sommier and Levier) (Apiaceae), and *Fallopia sachalinensis* (F. Schmidt) Nakai (Polygonaceae) (Michez et al., 2016) providing sufficient accuracies for operational application for the species except *F. sachalinensis*. The mapping of *Acacia saligna* (Labill.) H.L.Wendl (Fabaceae) on the Mediterranean Coast was also done using aerial photographs in conjunction with machine learning algorithms for a cheaper and efficient monitoring instrument (Marzialetti et al., 2021). In South Africa aerial photographs have been used to map *Pinus* spp. as they are invasive alien plants in mountains in the Western Cape (Forsyth et al., 2014). In recent decades, the advent of digital photography and Geographic Information Systems (GIS) has transformed aerial photography into a powerful tool for ecological and environmental studies (Morgan et al., 2010). Digital aerial images can be processed, shared, analyzed, and integrated with other spatial data in GIS platforms. This integration allows for more precise mapping, monitoring, and analysis of tree populations over time.

In South Africa a comprehensive survey to map the distribution and abundance of invasive alien plants (IAPs) was done using aerial photographs and extensive field data known as the "National Invasive Alien Plant Survey" (Kotzé et al, 2010). This was done as an attempt to develop and implement a cost-effective, objective, and statistically sound monitoring system for (IAP) species in South Africa, Lesotho, and Swaziland. Key environmental factors influencing the presence of IAP species were identified, including rainfall, soil depth, and the landscape's position (Kotzé et al., 2010). The research employed a unique sampling approach that strategically optimized sampling points along environmental gradients, particularly those that contributed most significantly to the occurrence of IAP species. The resulting maps can be used for past and future comparison of IAP invasion creating a monitoring system reliable at local scales more than 1:50 000 (Kotzé et al., 2010) making it successful in the mapping of 14 IAPs. However, many recent attempts have been made using high resolution hyperspectral imagery the most recent of which shows significant promise. In October 2024, the Agricultural Research Council released an Invasive Alien Tree Classification Map for the Cape floristic region. The project uses Sentinel-2 satellite imagery and a Random Forest machine learning classifier to create a 10-metre resolution map identifying six key invasive alien tree classes. A unique aspect of this project is its reliance on a "pure pixel approach". This method focuses on Sentinel-2 pixels that represent large, homogenous stands of each invasive taxon, ensuring greater accuracy in detecting areas of significant invasion. The programme boasts an over 90% accuracy rate of identifying invasive trees in the Biome with a few limitations (Rebello, 2024).

Geographic Information Systems

Geographic Information Systems are computer-based tools used to store, visualize, analyse, and interpret geographic data (Abdollahnejad and Panagiotidis., 2020). Geographic data identifies the geographic location of fixed objects, and this can be used to map tree populations (Olokeogun and Kumar, 2022). The geographical locations of trees can be used for mapping in tree management studies by utilizing GIS software such as Arc GIS and QGIS (Morgenroth and Visser, 2013). Geographic Information Systems have been used in forest ecosystem management and public park tree assessment (Pernar and Storga, 2005; Isa and Othman, 2012) for supporting management, planning and assessment of tree populations, as GIS can be used to create a geospatial tree information inventory system in urban park management (Othman et al, 2020).

Using aerial photographs in tracking population growth of *Salix babylonica* and *Salix fragilis* in South Africa

Salix babylonica L. (weeping willow) and *Salix fragilis* L. (crack willow) are two invasive trees commonly found in the Grassland biome of the Eastern Free State province in South Africa (Henderson, 1991). These trees play a detrimental role in the local ecosystem negatively influencing hydrological processes because they have a higher water consumption rate than native plants (Le Maitre et al., 2002). This excessive water uptake reduces the availability of water for plants and animals native to this riparian ecosystem (Le Maitre et al., 2002). The distinctive growth forms of these trees, particularly their height and canopy structure, combined with their tendency to invade open or disturbed areas and riparian zones, make them ideal candidates for historical analysis using aerial photography to track changes in distribution over time (Cobbing, 2006; Modipa, 2016). This method is particularly effective for identifying shifts in population dynamics and habitat colonization, as these willows frequently establish visible, homogenous stands that contrast with native vegetation.

Understanding the historical and current distribution of these species is essential for assessing ecological changes and informing conservation efforts. Historical aerial photographs compared to recent imagery offers an opportunity to compare past and present tree populations, providing insights into the invasive dynamics of these invasive trees overtime. In the case of invasions by *S. babylonica* and *S. fragilis* in the eastern Free State of South Africa, we have an opportunity to investigate these questions over a period of forty years using available aerial photographs. Answering these questions will help us understand the current distribution of *S. babylonica* and *S. fragilis* in the Grassland biome and inform future biodiversity conservation and ecosystem management as the species pose significant threats to native plant diversity and economic development (Henderson, 1991). Updating these data informs management practices, aids in updating risk analyses, and assists in the re-categorization of these species as needed; these species are currently listed as category 2 invaders under the Conservation of Agricultural Resources Act of 1983 (Henderson, 2001).

By georeferencing historical aerial photographs from 1984 onto contemporary satellite images in ArcGIS, this study aims to conduct a detailed analysis changes in the distribution patterns and population sizes of *S. babylonica* and *S. fragilis* within the Grassland biome from 1984 to 2023. This comparative approach will help identify distribution shifts over the past four decades.

Methods

Study species

The genus *Salix*, particularly *S. babylonica* and *S. fragilis*, exhibits distinctive physical traits that make them suitable for identification through aerial photography. *Salix babylonica* is characterized by its broad, weeping canopies and tall growth reaching heights of up to 25 meters—which can form dense patches along riverbanks and dam margins (Henderson, 1991). These large, pendulous branches create highly visible, compact canopies that are easily distinguishable from the surrounding vegetation in aerial imagery. *Salix fragilis* also grows into sizable trees, up to 20 meters tall, with its irregular crown and multi-trunked form contributing to its visibility in aerial surveys (Dirr, 2009). Both species are often found along riparian zones, agricultural margins, and roadside habitats, where their consistent presence in linear patterns alongside water bodies or roads can be detected and monitored using aerial photographs.

Study site

The study focused on assessing population size changes of willows in the Grassland biome in the Eastern Free State province, South Africa (Figure 3.1). This region is characterized by its diverse vegetation, with Poaceae, Asteraceae, and Fabaceae families being the most prominent (Dingaan and Du Preez, 2017), and the trees are mostly all invasive except for some native species such as *Leucosidea sericea* and the native *Salix* species *S. mucronate* Andersson (Saliaceae) (Ndamane et al., 2023). The area has experienced significant environmental changes over the past decades, such as woody plant density increasing with an estimated annual expansion of 0.22% between 1990-2013 within the Grassland biome (Skowno et al., 2017).

Figure 3.1: Distribution of the areas that historical images (grayscale images from 1984 within the rectangles) were used to assess the changes in willow populations over 40 years within the Eastern Free State, South Africa.

Aerial Photograph Data Collection

Historical imagery was obtained from the Cartographic Design and National Geospatial Infrastructure (CDNGI) Digital Portal (Figure 3.2). This portal provides an User Interface for clients to interact with geographical data and download material made accessible via this geoportal (https://ngi.dalrrd.gov.za/index.php/online-shop/what-is-itis-portal). Within this digital portal, an active map with areas in the Free State and Gauteng known to have *Salix* populations was selected. The primary objective was to locate suitable imagery from 1984 flown at a 1: 30 000 scale, resulting in an area of ~7km^2 per photograph. When potential images were identified, further checks were conducted to ensure that the images were captured during months when willows had foliage present and the image quality was sufficient, with minimal cloud cover to facilitate accurate species identification. This process proved to be highly time-consuming due to the limited availability of suitable imagery. Ultimately, 13 usable images were acquired through this method. It is important to note that these images were effectively random selections although dictated by image availability and quality.

Once downloaded, each image was opened in ArcGIS for further analysis. Firstly, images were georeferenced to their appropriate coordinates using ArcGIS geoprocessing tools. After georeferencing, a points shapefile was added into ArcGIS and was populated manually by zooming into historical images and selecting out *Salix* trees. Each point represented coordinates of each tree allowing for a count of each tree identified on the photographs. The coordinates were used for ground truthing, and the count was used to find the total number of trees on each site. Due to blurring near the edge of images potential trees at the edges were not included. The shapefiles created from the 1984 aerial photographs were superimposed onto the 2023 aerial images within the ArcGIS platform. This step allowed for direct visual and spatial comparison between the total of the points counted in 1984 and the points counted in 2023 between the two time periods for *S. babylonica* (Figure 3.3) and *S. fragilis* (Figure 3.4). Each set of aerial photographs (1984 and 2023) went through the process illustrated in (Figure 3.6).

Ground Truthing

To validate the findings, ground truthing was performed. This involved field visits to five of the 1984 aerial photograph selected during the aerial photography comparison, four to confirm *Salix babylonica* and one site to confirm *Salix fragilis*. At each site *Salix* species were confirmed as the dominant species and no misidentification were made.

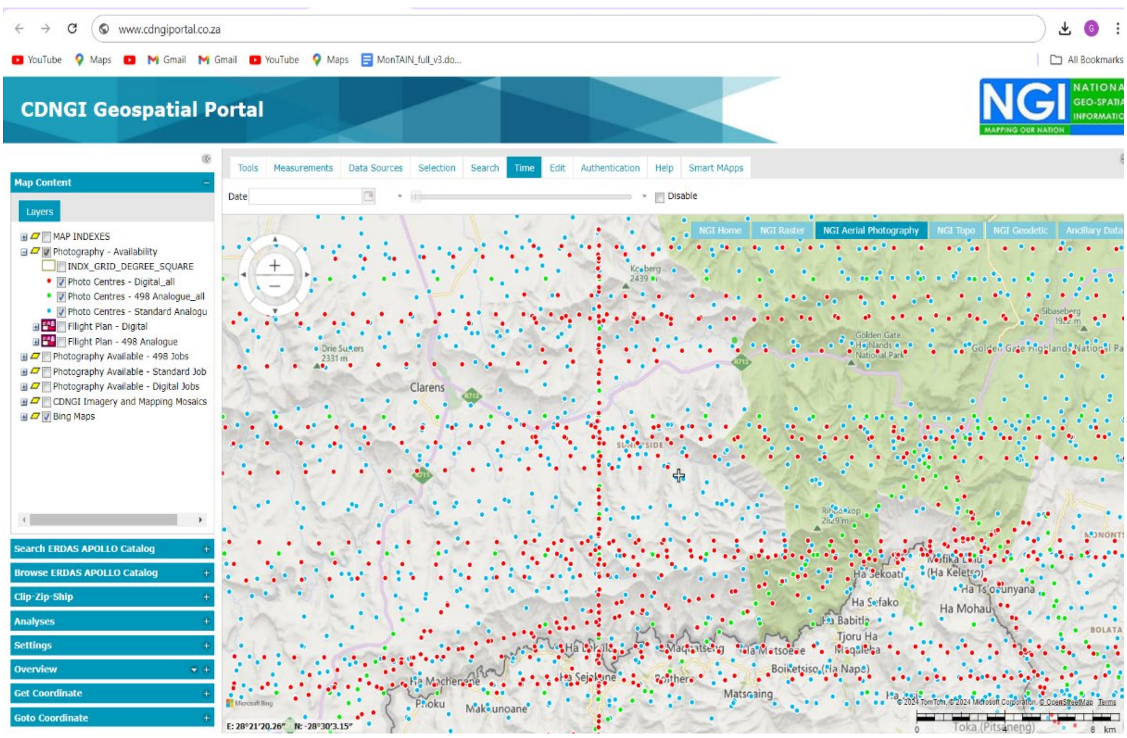

Figure 3.2: CDNGI geospatial portal showing available imagery. Red and blue points represent an available aerial digital photograph of that location on historical imagery. Clicking on each point will display information (e.g., location details, time of event and description of the incident) and most importantly the link to the digital photograph through *CDNGI Geospatial Portal.* Retrieved from http://www.cdngiportal.co.za/CDNGIPortal/.

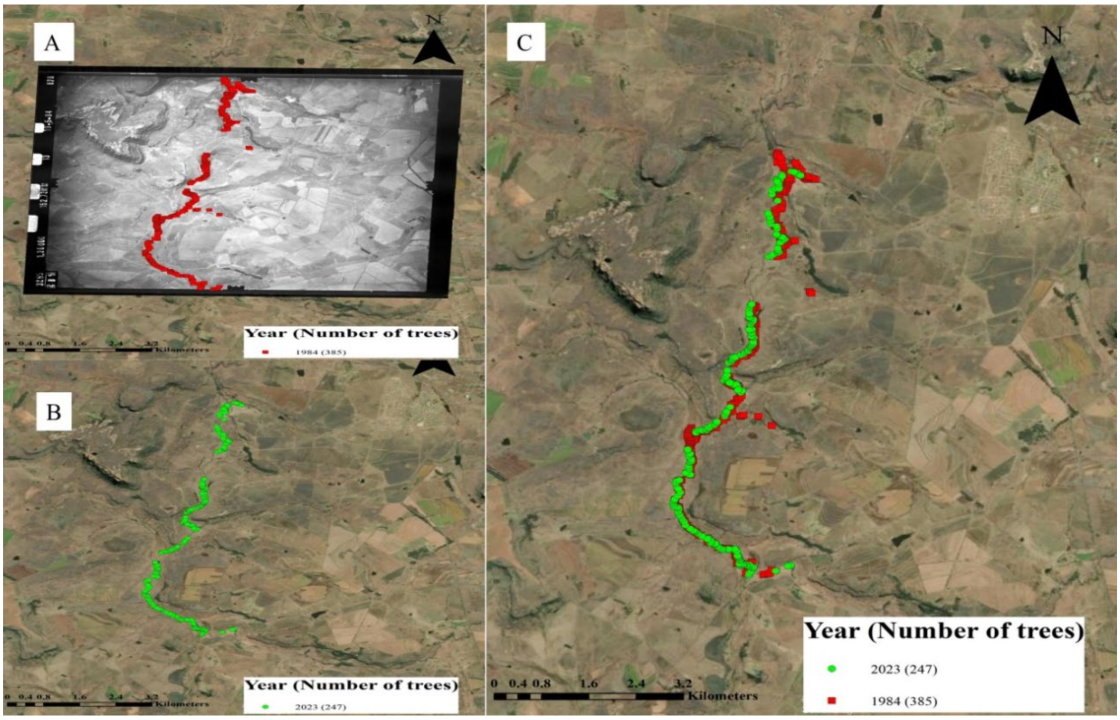

Figure 3.3: (A) Red points showing the presence of *Salix babylonica* in Makweteng, Free State in 1984 (B) Green points showing the presence of *Salix babylonica* in Makweteng, Free State South Africa in 2023. (C) A superimposed visual of the points showing the presence of *Salix babylonica* in Makweteng, Free State, South Africa comparing 1984 and 2023 populations.

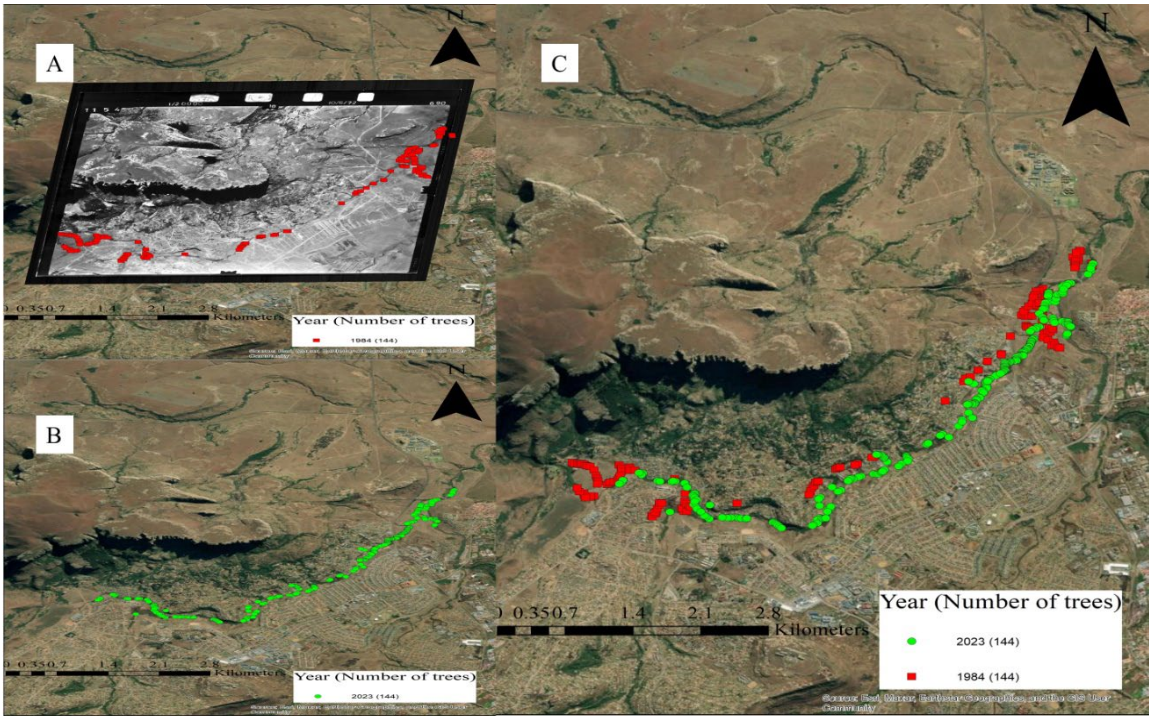

Figure 3.4: (A) Red points showing the presence of *Salix fragilis* in Qwaqwa, Free State in 1984 (B) Green points showing the presence of *Salix fragilis* in Qwaqwa, Free State South Africa in 2023. (C) A superimposed visual of the points showing the presence of *Salix fragilis* in Qwaqwa, Free State, South Africa comparing 1984 and 2023 populations.

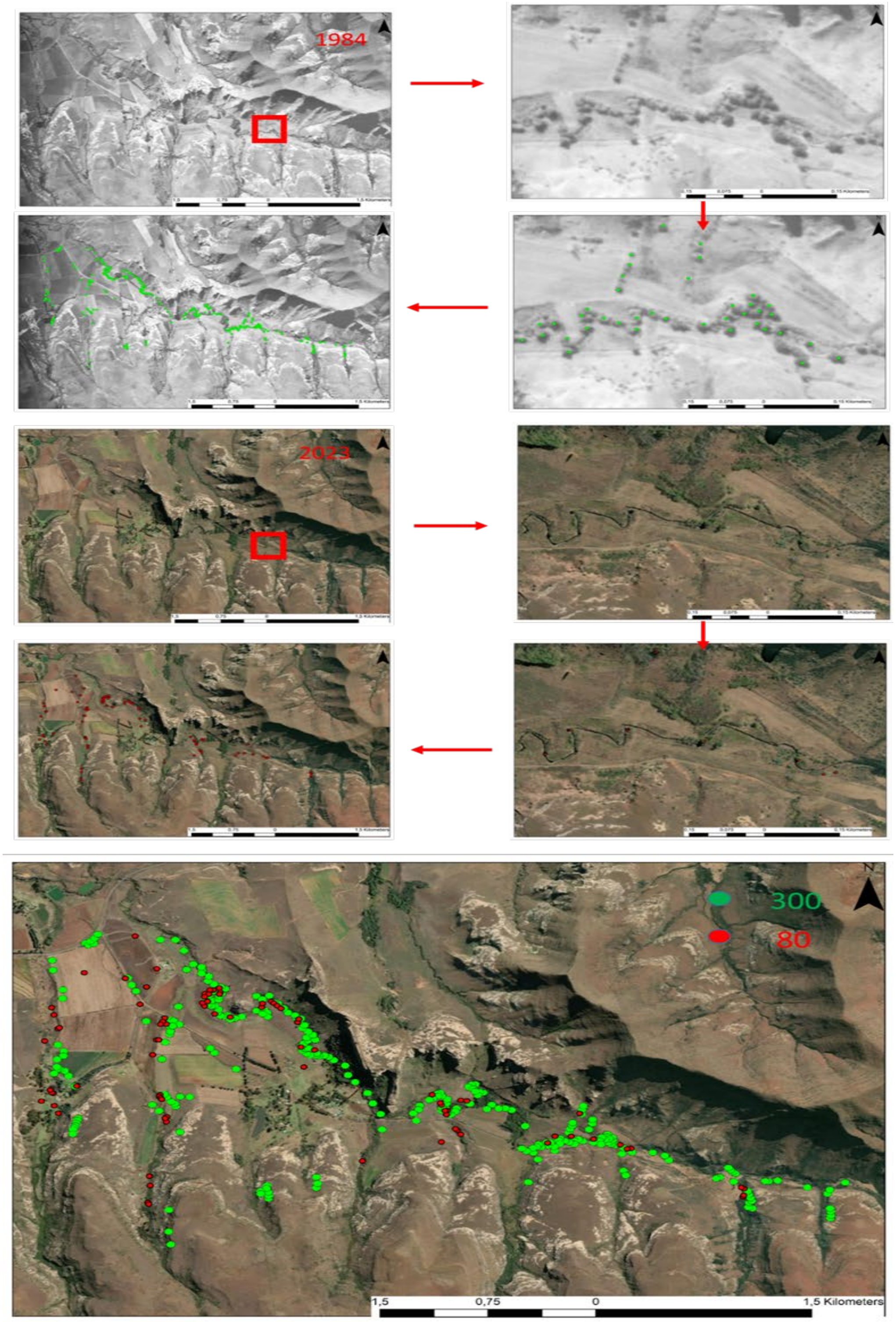

Figure 3.5: Illustration of the process of plotting points, each representing a *Salix* tree, within each image on historical imagery as well as the current aerial view of same spatial area as the historical imagery. Red points showing the presence of *Salix* trees in 1984 and green points showing the presence of *Salix* trees in 2023.

Statistical Analysis

The number of trees per site (population size) of *S. babylonica* and *S. fragilis* in 1984 and 2023 were tested for normality using a Shapiro-Wilks test. *Salix babylonica* population sizes were not normally distributed (P = 0.04) and were log-transformed, which resulted in them becoming normally distributed (P = 0.11). Data from *S. fragilis* were normally distributed (Shapiro-Wilks test, P = 0.33). All variances were equal (Fisher's Ratio test, P > 0.05). Number of trees per population in 1984 and 2023 for *S. babylonica* (log-transformed data) and *Salix fragilis* were compared with a paired t-test with equal variances. Analyses were conducted in R (Version 4.3.1; R Core Team, 2023). Data are presented as mean ± standard error.

Results

Salix babylonica

The population sizes of *S. babylonica* were compared between 1984 and 2023 (Figure 3.6). In 1984, the mean population size per 7km^2 (the area covered by an aerial photograph) was 454 ± 66 trees, whereas in 2023, the mean population size was 281 ± 70 trees. There is a statistically significant decrease in population size of *S. babylonica* between 1984 and 2023 (t_{12}=3.46 P = 0.005).

Salix fragilis

The comparison of *Salix fragilis* populations between 1984 and 2023 yielded different results (Figure 3.7). In 1984, the mean population size per 7km^2 (area covered by an aerial photograph) was 426 ± 282 trees, while in 2023, the population size increased slightly to 539 ± 297 trees. Although small sample sizes were small (n = 2 for both years), a paired t-test indicated no significant difference in *S. fragilis* population sizes between 1984 and 2023 (t_1= -7.53, P= 0.084).

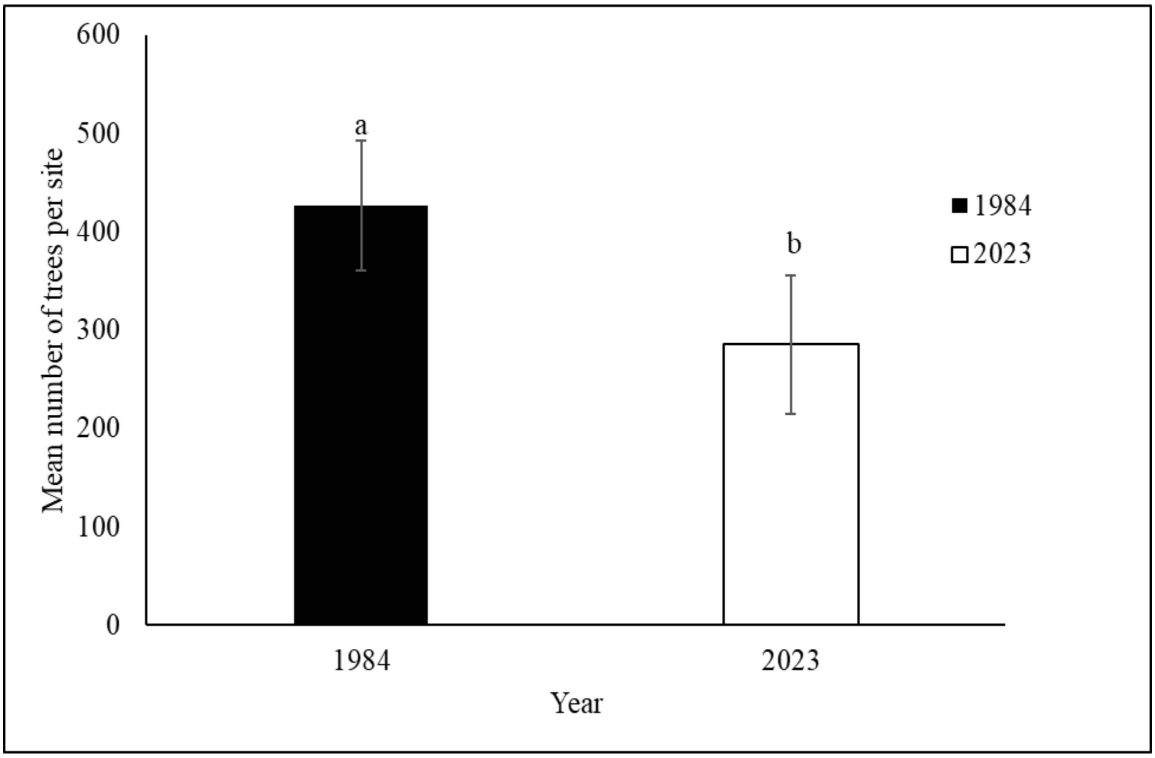

Figure 3.6: Number of *Salix babylonica* trees (mean ± standard error) in 1984 and 2023 per site (n = 11 sites) using aerial photographs in the Eastern Free State province, South Africa. Different letters above bars denote significant difference ($P < 0.05$).

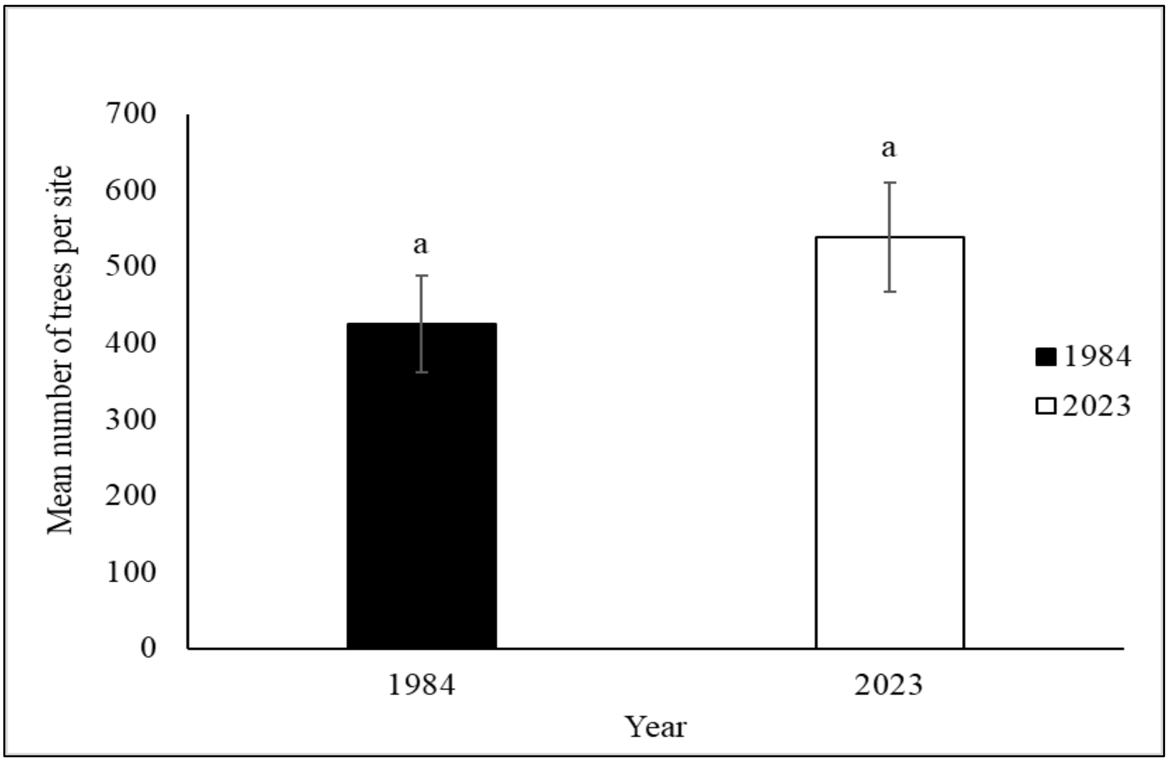

Figure 3.7: Number of *Salix fragilis* trees (mean ± standard error) in 1984 and 2023 per site (n = 2 sites) using aerial photographs in the Eastern Free State, South Africa. Letters above the bars represent significant differences; there is no significant difference in population sizes between years ($P > 0.05$).

Discussion

The comparison of *S. babylonica* populations from 1984 to 2023 revealed significant changes in the tree distribution over the 40-year period. Results indicate a statistically significant reduction in the population sizes of *S. babylonica* between the two time periods, with the mean population in 1984 being substantially higher than in 2023. This decline could be attributed to several factors, including environmental changes, land use modifications, or shifts in water availability, which are critical for the survival of riparian species like *S. babylonica*. These findings align with some studies on plant species dynamics, where shifts in water regimes often contribute to significant population declines (Chamier et al., 2012). This reduction suggests that factors such as no recruitment may be contributing to the decline of this species in certain areas. In Chapter 2, it was noted that there are extremely low levels of recruitment for *S. babylonica* with almost 80% of the trees recorded being mature trees showing signs of old age.

In contrast, *Salix fragilis* populations have shown no significant change over the same period, with population sizes remaining stable over the past 40 years. This species' preference for riparian zones (Henderson, 1991; Henderson, 2007), where conditions are more stable and water availability is higher, likely contributes to its continued survival and spread. The ability of *S. fragilis* to form pure stands near water bodies poses a particular concern for native biodiversity, as it can alter riverbank ecosystems and outcompete indigenous species adapted to these environments (Kennedy et al., 2003).

The use of aerial photography and GIS in this study has proven invaluable for detecting changes in trees populations over time. By analyzing historical and current tree populations, these tools have provided insights into the spatial extent and dynamics of the *Salix* invasions. The ground-truthing exercises further corroborated the aerial data, reinforcing the validity of the observed patterns. Despite these results obtained from the ground truthing, in person observations noted that other *Salix* trees were present, suggesting the sites were mixed stands (Figure 3.8). Within a *S. fragilis* site, *S. babylonica* was observed suggesting that even with a randomized way to choose locations to visit one could miss these anomalies.

The contrasting trends between the two willow species suggest a shift in management priorities may be necessary. Since *S. babylonica* is in decline, it may no longer require intensive management interventions as the most invasive alien in the riparian areas (Henderson, 2007). Instead, *S. fragilis* represents a more immediate concern due to its increasing density and potential to disrupt ecosystems, particularly near rivers and watercourses. The reduction of *S. babylonica* is a positive development for water systems, as fewer invasive willows may lead to improved water availability and healthier ecosystems. However, the increasing presence of *S. fragilis* could counteract these benefits if left unchecked, necessitating a focused management effort. Currently, willows are not listed under the National Environmental Management: Biodiversity Act (NEMBA) only under the Conservation of Agricultural Resources Act of 1983 (CARA). Given the increasing population sizes of *S. fragilis* and its potential ecological impact, it may be prudent to reconsider this. The inclusion of *S. babylonica* and particularly *S. fragilis* under these frameworks could provide legal support for their control and ensure that management practices are implemented to stop their spread.


Figure 3.8. *Salix babylonica* (inside red circles) within a stand comprised mostly of *Salix fragilis* in Clarens, Free State, South Africa. (Photograph: T. Mashamba).

Conclusions

This study aimed to compare the past and present distribution patterns of *Salix babylonica* and *S. fragilis* in the Grassland biome of the Eastern Free State, South Africa, over a 40-year period (1984-2023). By utilizing historical and contemporary aerial photographs, combined with Geographic Information System techniques, a detailed analysis of the changes in population size and distribution of these invasive willow species was provided. The findings indicate a significant decrease in the presence of *S. babylonica* between 1984 and 2023. This reduction suggests that factors such as little to no recruitment may have contributed to the decline of this species in certain areas. Conversely, *S. fragilis* demonstrated more stability in its distribution, particularly in riparian zones, where water availability remains consistent. These results have important implications for the management of invasive species in the region especially the persistence of *S. fragilis* in key areas highlights the need for ongoing monitoring and targeted control strategies, particularly near water bodies where the species continues to thrive.

Additionally, this study shows the effectiveness of GIS in monitoring long-term ecological changes within a grassland environment, providing valuable insights into species distribution dynamics and habitat transformation over time. The ability to compare historical and contemporary data provides valuable insights into the dynamics of invasion, which can inform future conservation and management efforts even with limitation pixel resolutions in excess of 2-3 meters which reduces the ability to interpret the aerial photo with certainty. Therefore continued use of these techniques, coupled with ground truthing and field surveys, will enhance our understanding of invasive species and support biodiversity conservation. In conclusion, the integration of historical data with modern GIS tools has proven to be a valuable approach for assessing the spread of invasive species. By understanding the historical and current distribution of *S. babylonica* and *S. fragilis*, we can contribute to more effective management practices and the preservation of native biodiversity in South African ecosystems.

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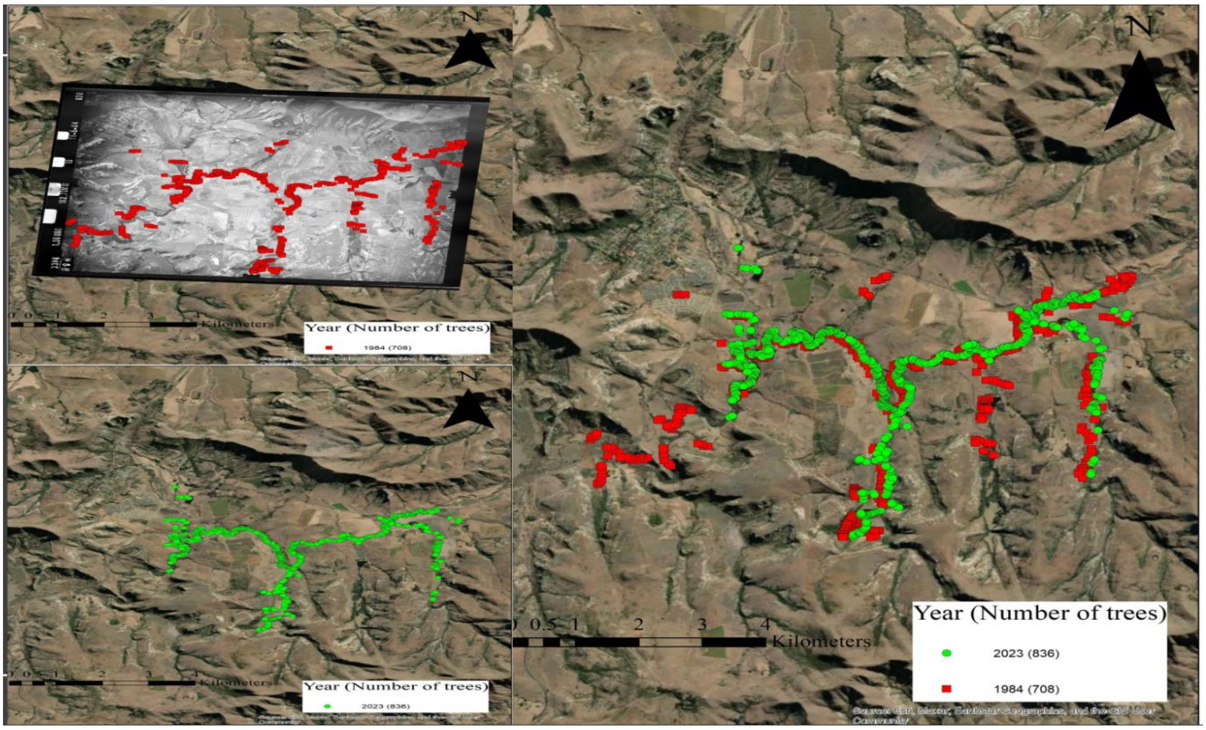
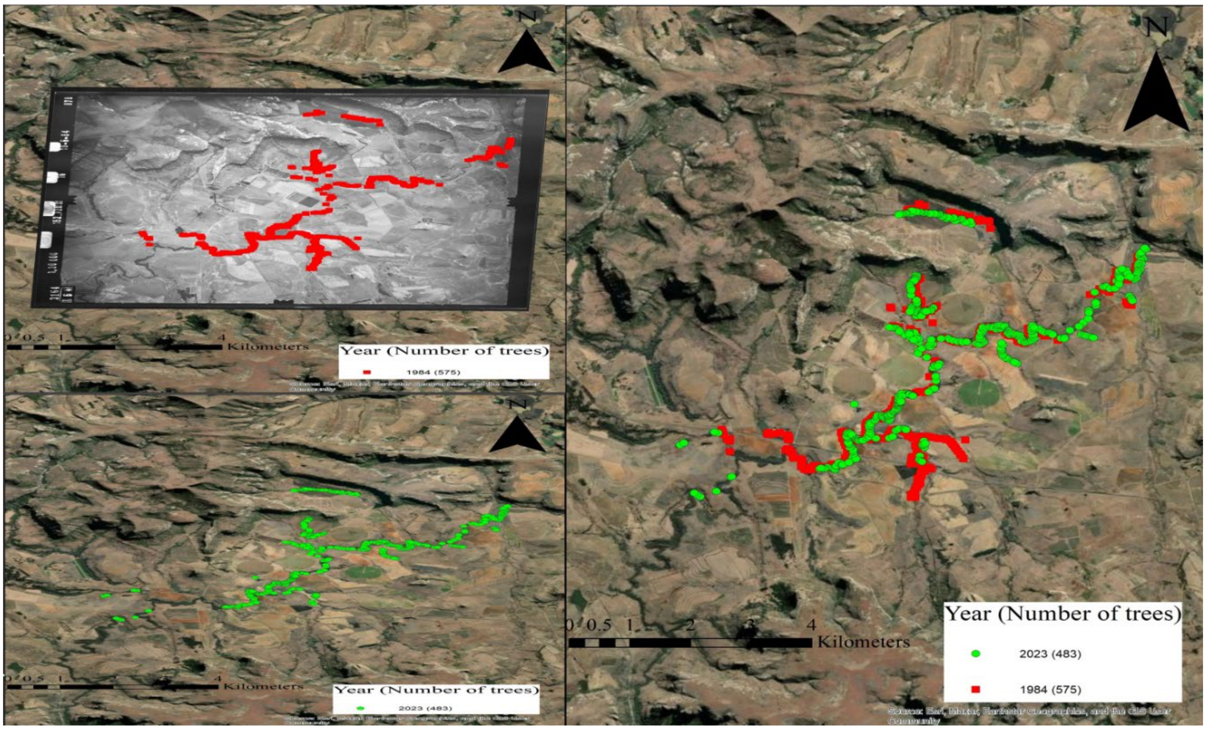
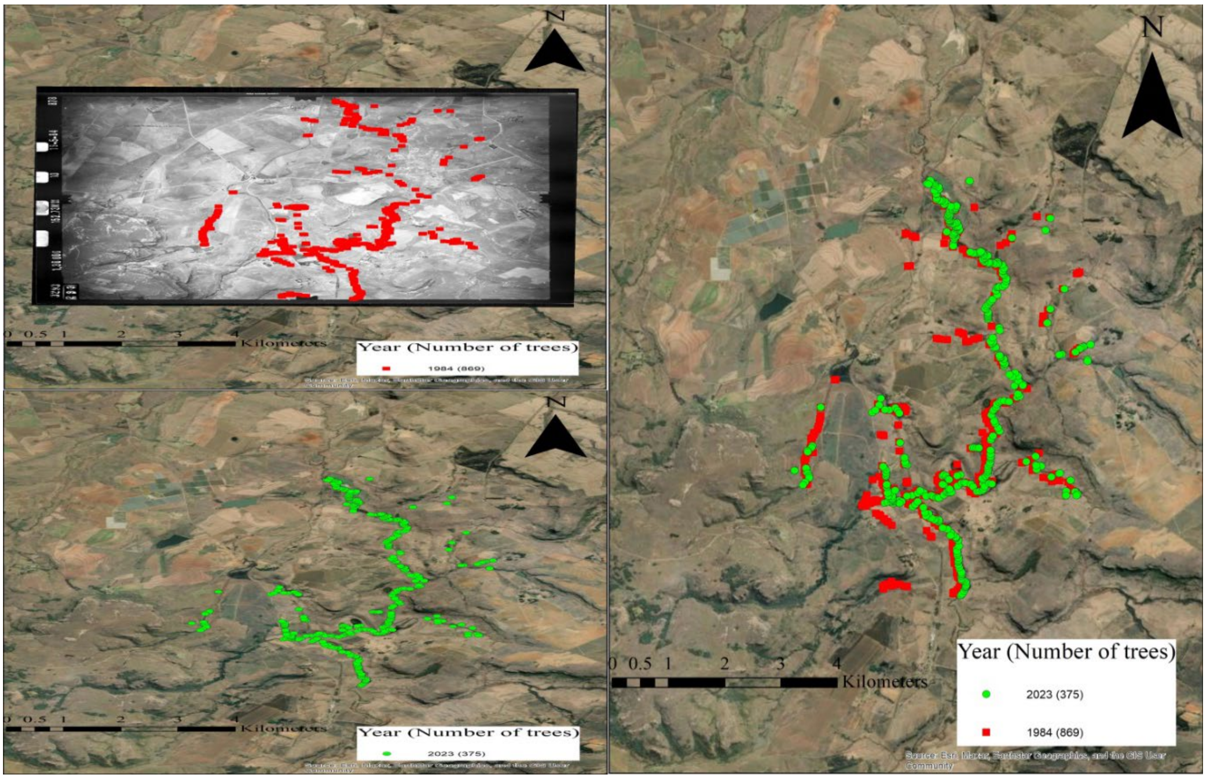
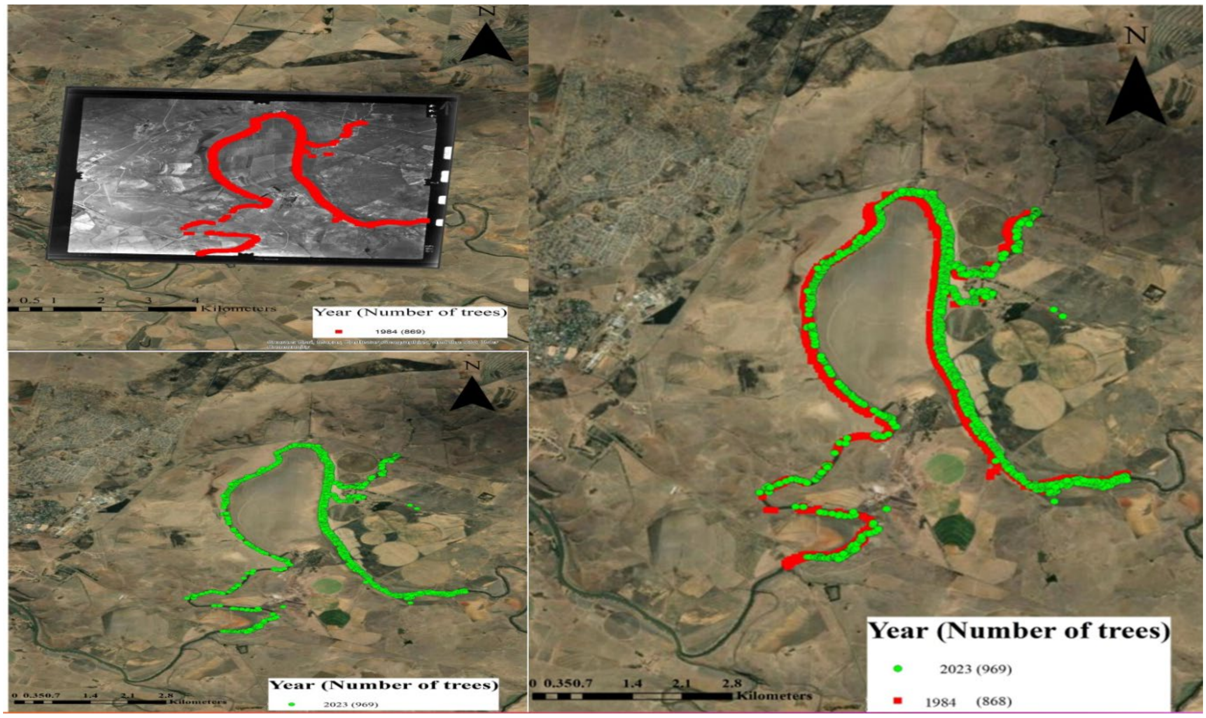
Appendix

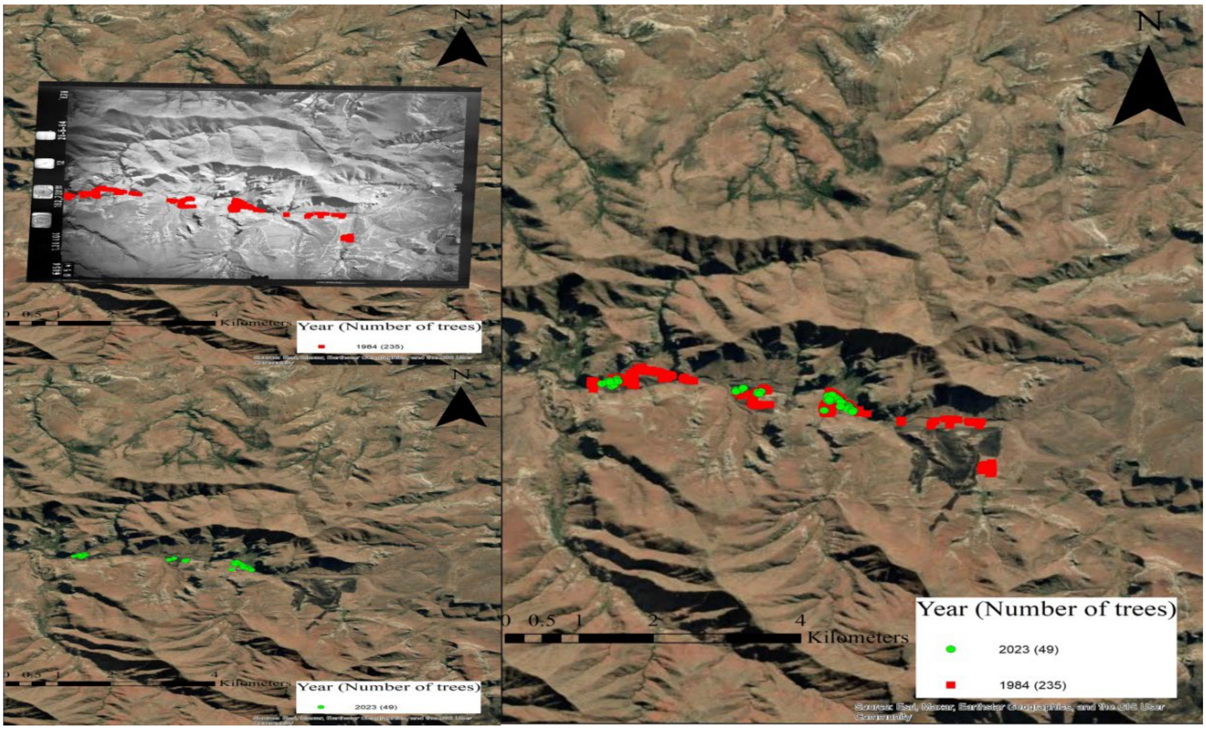
Images of the 13 sites that were compared aerially between 1984 and 2023

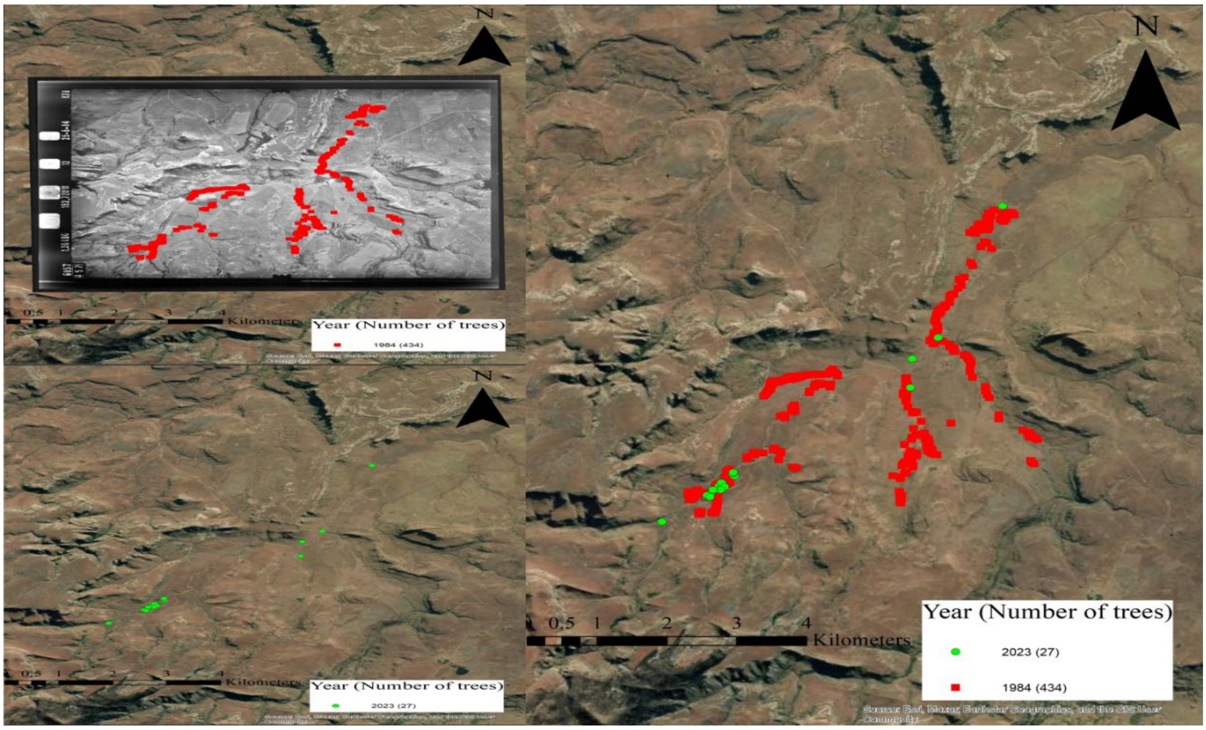


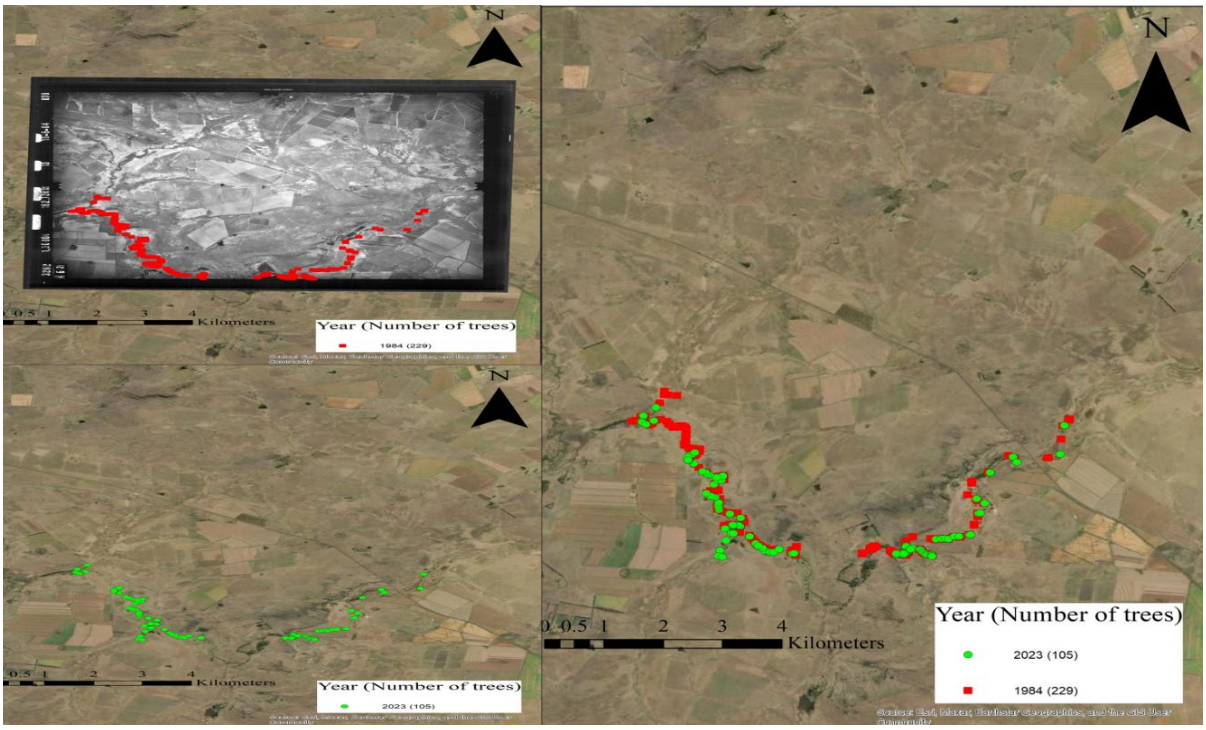


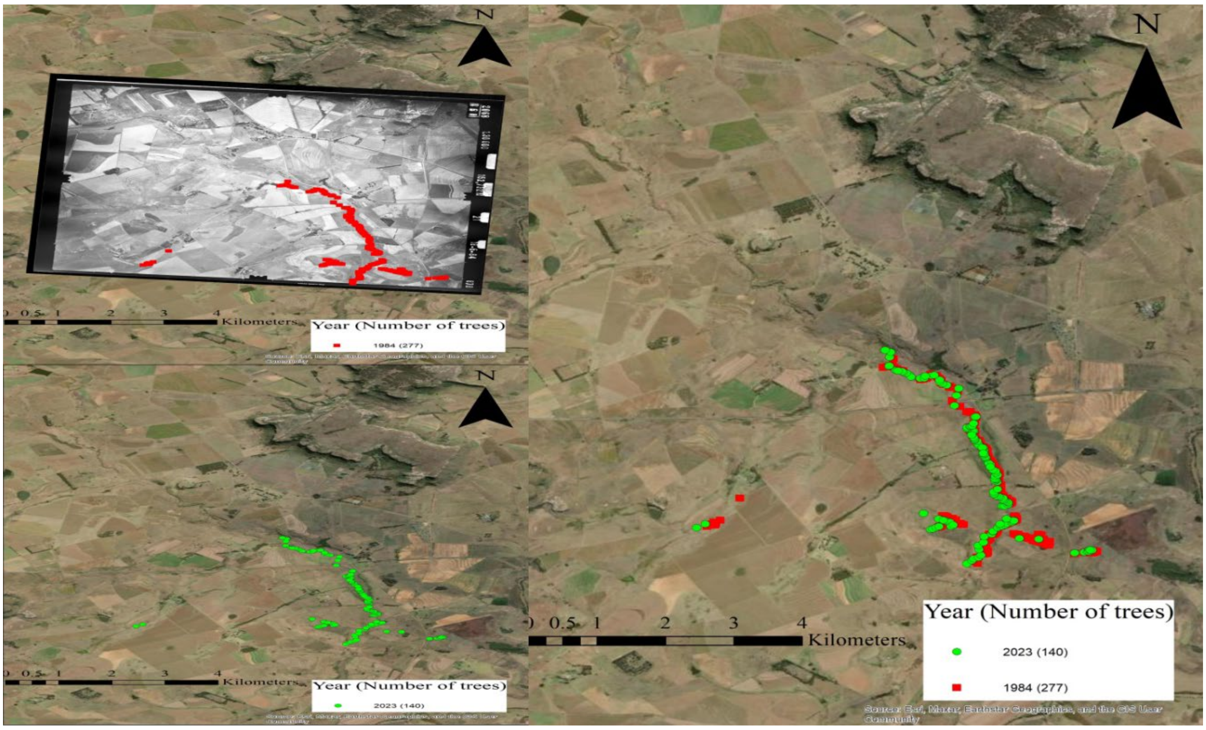

N
0 0.5 1 2 3 4 Kilometers
Year (Number of trees)
1984 (869)
2023 (375)
Year (Number of trees)
2023 (375)
1984 (869)

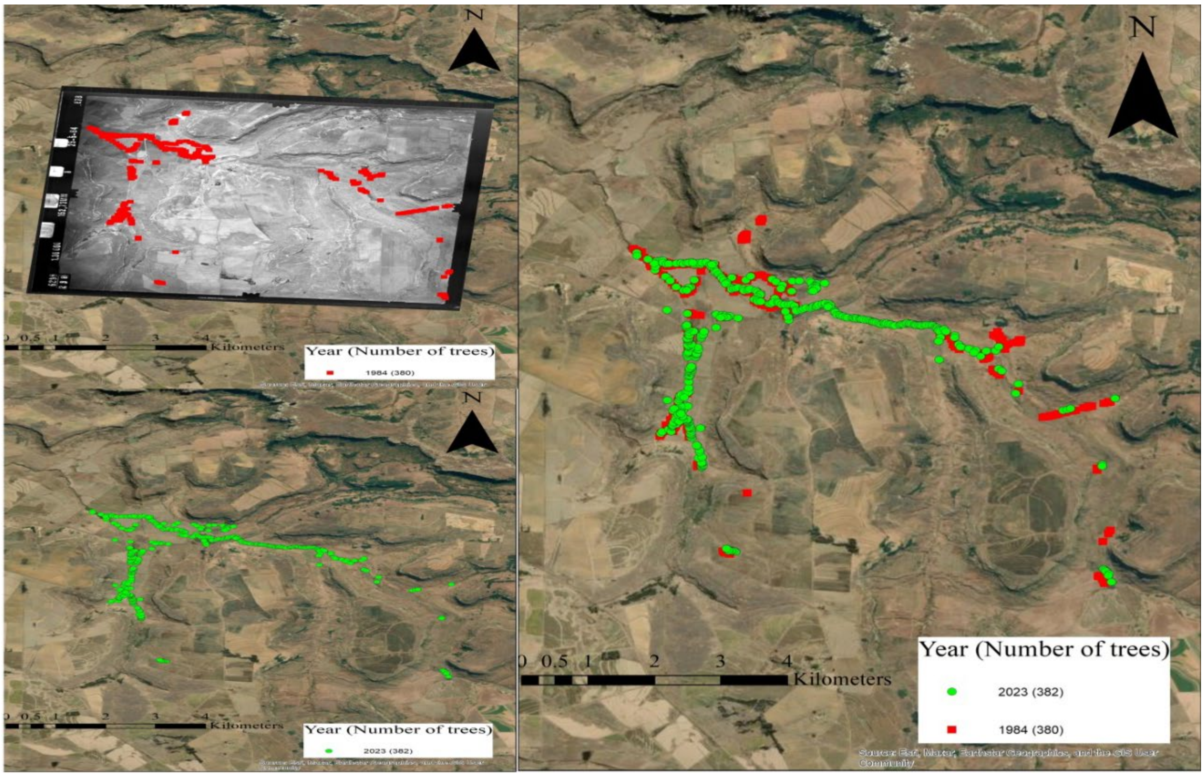

N
Year (Number of trees)
1984 (869)
Year (Number of trees)
2023 (969)
0 0.350.7 1.4 2.1 2.8 Kilometers
Year (Number of trees)
2023 (969)
1984 (868)

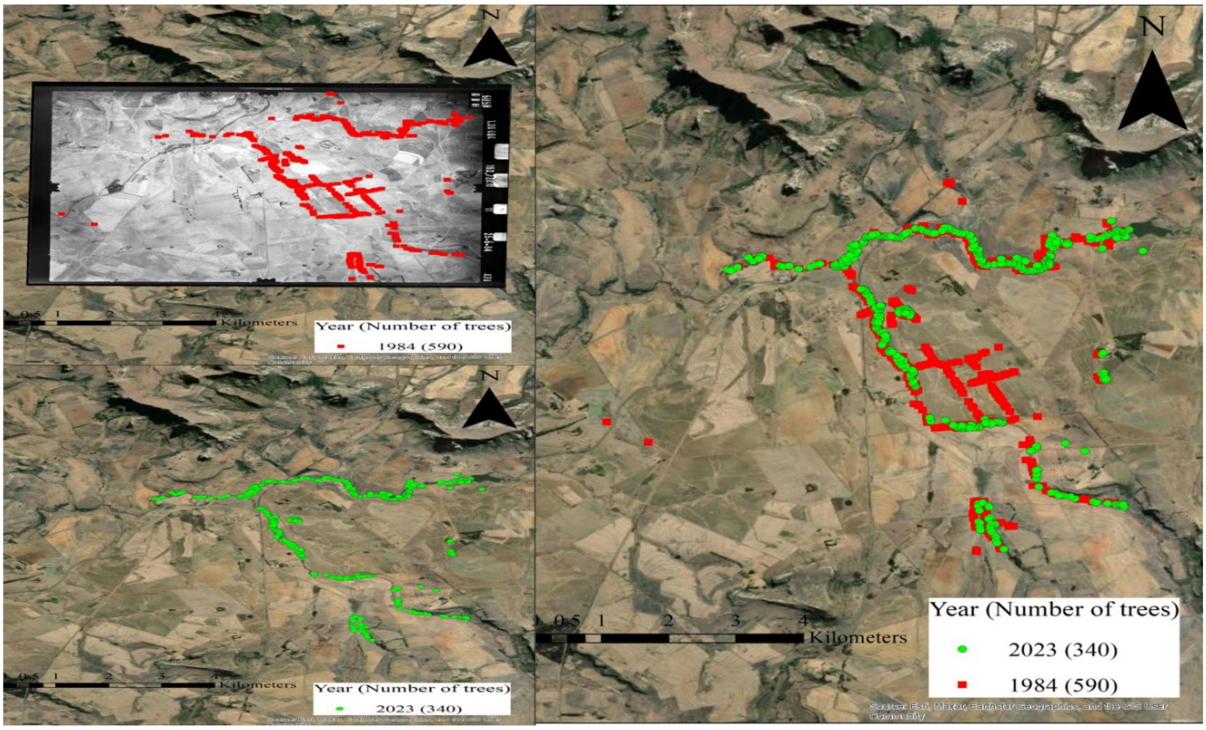

N
Year (Number of trees)
1984 (235)
2023 (49)
0 0.5 1 2 4
Kilometers
Year (Number of trees)
2023 (49)
1984 (235)
Source: Esri, Maxar, Earthstar Geographics, and the GIS User Community

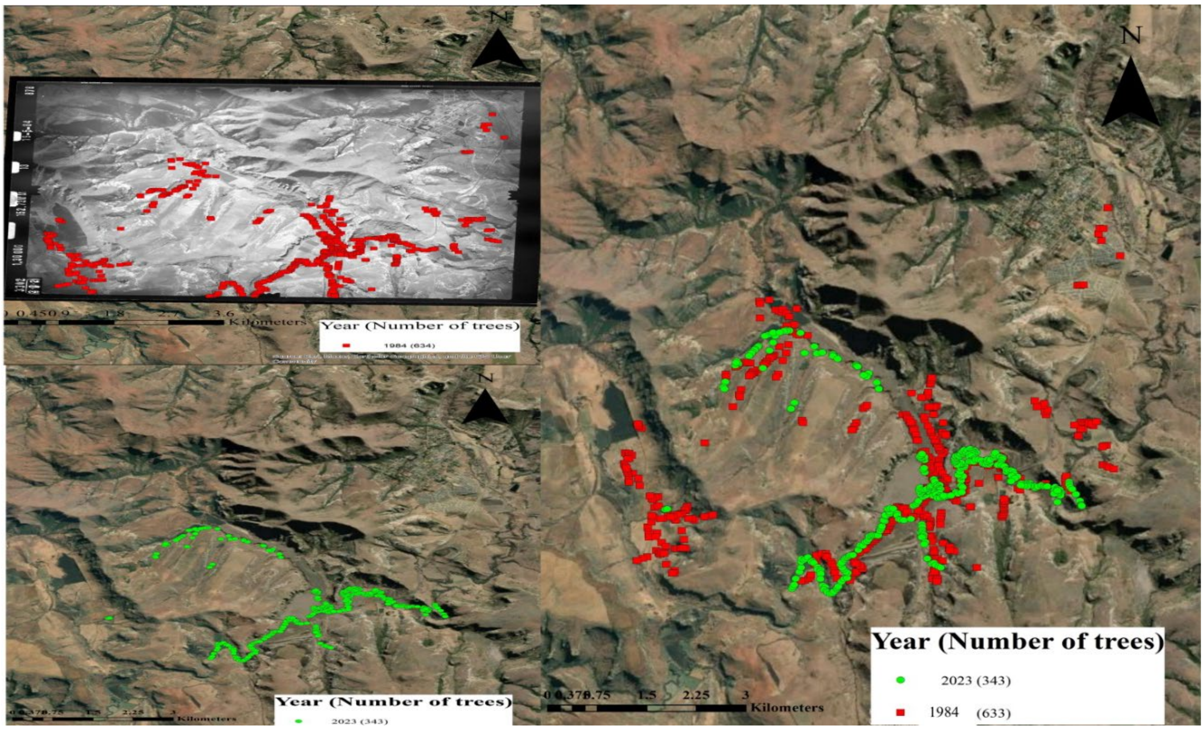

N
Year (Number of trees)
1984 (434)
2023 (27)
0 0.5 1 2 3 4
Kilometers
Year (Number of trees)
2023 (27)
1984 (434)

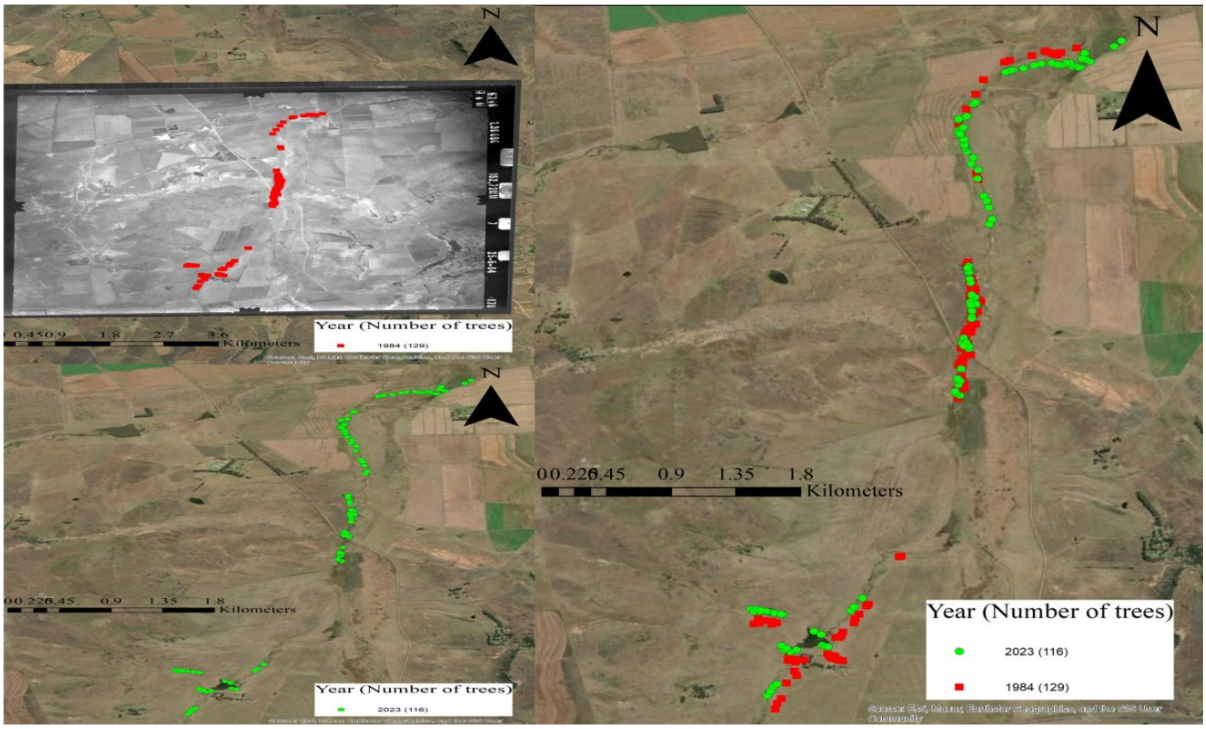

N
0 0.5 1 2 3 4 Kilometers
Year (Number of trees)
1984 (229)
2023 (105)


N
0 0.5 1 2 3 4 Kilometers
Year (Number of trees)
1984 (277)
2023 (140)


N
Kilometers
Year (Number of trees)
1984 (380)
2023 (382)
0 0.5 1 2 3 4
2023 (382)
1984 (380)


N
Kilometers
Year (Number of trees)
1984 (590)
2023 (340)
0 0.5 1 2 3 4
2023 (340)
1984 (590)


N
Year (Number of trees)
1984 (634)
Kilometers
N
Year (Number of trees)
2023 (343)
N
0 0.375 0.75 1.5 2.25 3
Kilometers
Year (Number of trees)
2023 (343)
1984 (633)


N
Year (Number of trees)
1984 (129)
0 0.45 0.9 1.8 2.7 3.6
Kilometers
N
0 0.225 0.45 0.9 1.35 1.8
Kilometers
Year (Number of trees)
2023 (116)
N
0 0.225 0.45 0.9 1.35 1.8
Kilometers
Year (Number of trees)
2023 (116)
1984 (129)

CHAPTER 4

INSECTS ASSOCIATED WITH *SALIX BABYLONICA* AND *SALIX FRAGILIS* IN THE GRASSLAND BIOME OF THE EASTERN FREE STATE, SOUTH AFRICA

Introduction

There are an estimated 450 species of willow (*Salix* spp., family Salicaceae) globally, predominantly distributed in the temperate zones of Asia, Europe, and North America (Dickmann and Kuzovkina, 2014). In North America, willow species are prevalent across Canada, the United States and Mexico. Notable species in Asia, especially in China's temperate region, include *Salix nigra* Marshall (black willow), *S. alba* L. (white willow), and *S. babylonica* L. (weeping willow) (Wagner et al., 2021). In Europe, willows thrive from Scandinavia to the Mediterranean, with common species such as *S. alba*, *S. fragilis* L. (crack willow) and *S. cinerea* L. (grey willow) (Wagner et al., 2021). Of these species, *S. fragilis* and *S. babylonica* have invaded the Grassland biome of South Africa (Henderson, 1991). *Salix babylonica* was noted by Henderson (2007) as the third most prominent Invasive Alien Plant (IAP) in riparian parts of this biome. *Salix fragilis* was observed to be invading this biome with a lower prominence value compared to *S. babylonica* (Henderson, 2007). Furthermore, surveys indicated that *S. babylonica* is the most dominant IAP in most riparian and wetland habitats, with 1,323 occurrence records in the Southern African Plant Invaders Atlas (SAPIA) database in the late 2000's, in stark contrast to the 175 occurrence records for *S. fragilis* at the time (Henderson, 2007).

Willows in their native environments around the world provide habitats and food for several organisms. Large mammals browse on their foliage and shoots, and birds excavate holes for nests (Charles et al., 2014). However, insects have the greatest impact as they attack all parts of the tree. Insects play a significant role in the ecology of *Salix* species, both as pests and pollinators. A significant number of pests have been identified on Salicaceae species in their native ranges in Asia (Pandey et al., 2007) and Iran (Akbarian et al., 2006; Babmorad et al., 2006). Salicaceae species are attacked by at least 300 species of insects and mites in North America (Mattson et al., 2001) and 525 species in Europe (Delplanque, 1998). Over 80 insect species are associated with willows in Lithuania, of which 17 are considered harmful to commercial plantations of *Salix* (Noreika and Smaliukas, 2005). These insects also attack the common willow species and their hybrids: *S. alba* L., *S. molissima* L., *S. viminalis* L., *S. dasyclados* Wimm., *S. purpurea* L., and *S. integra* × *S. kochiana* (Shaburov); as well as 84 other forms, cultivars, and clones in the region (Noreika and Smaliukas, 2005). On these trees the insects eat the leaves and buds, suck sap, induce galls, bore into shoots and wood, attack roots and transmit plant diseases (Charles et al., 2014).

Several Salicaceae species have been introduced to many regions outside of their native ranges through human activities (Kueffer, 2017). The insects found on these trees are often restricted to a few species that arrived with the plants themselves (Charles et al., 2014), and only a small number of these species regularly cause such severe physical damage to the trees to be called pests. For example, the leaf beetle, *Chrysomela lapponica* L. (Chrysomelidae), which can be present in large numbers on its host plant in northern Europe (Gross et al., 2007), is considered a pest as it causes defoliation of willows (Zvereva et al., 1995).

Insect pests of *Salix*

Insects can significantly affect willows through various mechanisms. They usually eat buds, leaves and shoots of willow trees with the most severe attacks leaving the trees defoliated, resulting in declining growth rates and even death of the trees (Charles et al., 2014). Gall-inducing insects influence willow physiology and morphology, creating characteristic gall-structures to dwell in (Hartley, 1992; Nyman and Julkunen-Tiitto, 2000; Charles et al., 2014). Wood-boring insects can weaken trunks, while leaf miners damage leaves and cause premature leaf drop (Charles et al., 2014). The mechanisms of gall formation and plant manipulation are still being studied, with some similarities observed between gall-inducers and leaf-miners (Giron et al., 2016). Insects may also spread plant infections. Plant viruses, such as carrot red leaf virus and carrot mottle virus, are spread by *Cavariella aegopodii* (Hemiptera: Aphididae), also known as willow-carrot aphids (Jiao et al., 2024). This aphid is a sap-sucking insect widely distributed in Asia, Australia, Europe and North America. The deleterious effects of *C. aegopodii* on plant growth primarily stem from its feeding activities and its role as a vector (Jiao et al., 2024).

The willow sawfly *Nematus oligospilus* (Diptera: Tenthredinidae), inadvertently introduced to Australia and southern Africa, is a leaf miner attacking invasive willows (*Salix* spp.) (Urban and Eardley, 1995; Caron et al., 2011). *Nematus oligospilus* feeds and completes its life cycle almost exclusively on *Salix* species, from laying of eggs on the top or lower surfaces of leaves to consumption of the leaves by the larvae. The larvae can consume entire leaves leaving only the major and secondary veins (de Tillesse et al., 2017). Minor feeding damage has been observed on other species in the Salicaceae family, such as *Populus nigra* L. (Caron et al., 2011). In southern Africa, the sawfly does not attack the indigenous willow *S. mucronata* Andersson (Salicaceae) or the economically important *Populus deltoides* W.Bartram ex Marshall (Urban and Eardley, 1995).

The interaction between plant species and associated insect populations is a crucial component of ecosystem dynamics. In the Eastern Free State, the invasive *S. babylonica* and *S. fragilis* have a negative impact particularly in riparian zones (Henderson, 2007). These species provide habitat and food resources for various insect species (Ostaff et al., 2015; Bertrand et al., 2019), which could be investigated in a future search for potential biocontrol agents of invasive willows in South Africa.

The aim of this chapter was to investigate the diversity and abundance of insect species that interact with *S. babylonica* and *S. fragilis* within the Grassland biome of the Eastern Free State. The study assessed the ecological roles of these insects from literature, to understand how these insects may be affecting tree health and reproduction of willow populations in the eastern Free State. This aim was achieved through the following three objectives: (i) identifying and classifying the invertebrate species associated with *S. babylonica* and *S. fragilis* in selected study sites within the Grassland biome; (ii) identifying the potential impact of these invertebrates on the health, growth, and reproduction of the willow populations, with the use of literature; and (iii) assessing whether there are differences in invertebrate communities between *S. babylonica* and *S. fragilis*.

Materials and Methods

Study site and species

Thirteen sites were chosen for this study, located between Harrismith and Clarens, stretching between the Maluti-a-Phofung Local Municipality and Dihlabeng Municipality in the Eastern Free State, South Africa (between coordinates 28°33'04"S 29°04'48"E and 28°21'41"S 28°17'42"E; Figure 4.1). Invertebrates associated with one to four mature *S. babylonica* and/or *S. fragilis* trees were sampled once at each site between 08h00 and 14h00 over four days (15, 16 and 17 December 2023 and 4 January 2024, resulting in a total of 40 trees of each *Salix* species sampled.

Sampling Methods

Invertebrates on the foliage of willow trees were collected using a sweep net (38.1 cm diameter collapsible net) by placing the net over low-hanging branches and vigorously shaking the net in figure-eight motions for 10-15 seconds per branch. This was repeated five times per tree, using different branches for each replicate sample of a tree. All sites where trees were sampled were riparian in nature with some of the sites located on roadsides. Four sites in Clarens were located on farms, two in a residential area in Harrismith, and one near a small dam on the grounds of the University of the Free State's Qwaqwa campus. The remaining six sites were located on the banks of the Metsi-matsho River, Phuthaditjhaba. All collected invertebrates were transferred to screw-top containers filled with 70% ethanol. Insect and arachnid samples were sorted into families and morphospecies using guidebooks and expertise in the Department of Entomology and Zoology (University of the Free State). Insects of importance (high abundance or multiple collections) were sent to the National Collections of Insects – Agricultural Research Council – Plant Health and Protection (ARC-PHP) Biosystematics section for full species identifications. During this survey galls were observed on a proportion of the sampled trees. Photographs were taken of any galls observed and the percentage of these was noted.

Cumulative species curves for invertebrates collected from each tree species were generated over sites, and analysed using regression analysis, to assess sampling effort. All graphs and analyses were performed using Microsoft Excel.

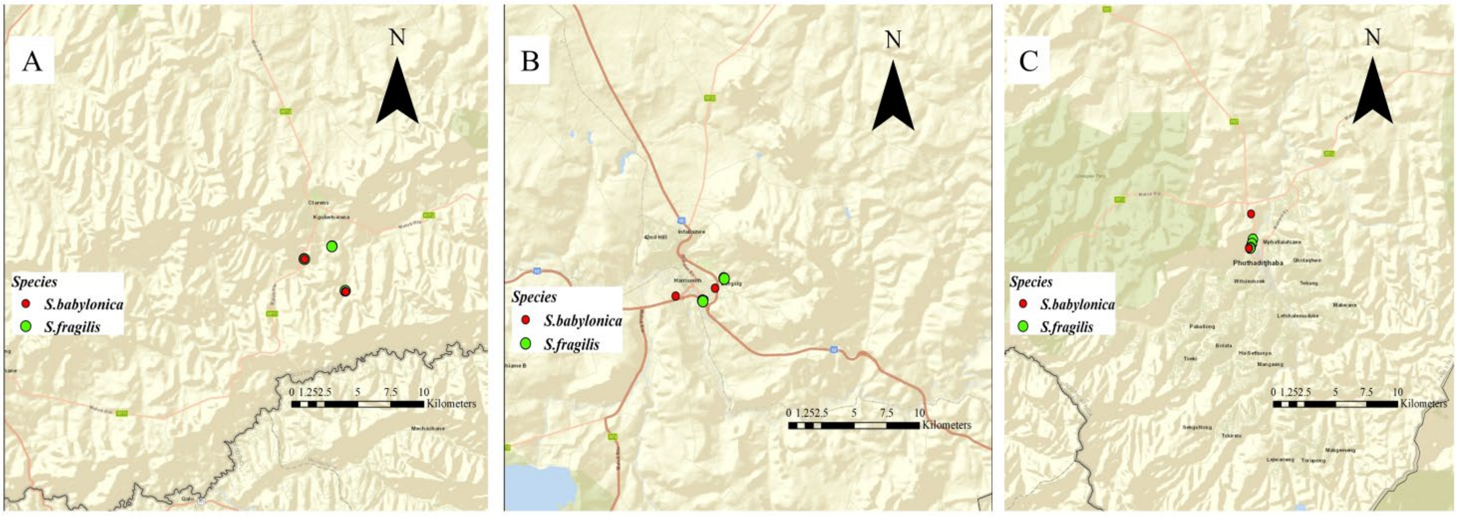


Figure 4.1: Location of the 13 collection sites of insects associated with *Salix babylonica* and *S. fragilis* spread across (A): Clarens, (B): Harrismith and (C): Phuthaditjhaba in the eastern Free State, South Africa.

Results

A total of 348 insects representing 21 families, and one mite from the order Acari (arachnid), were collected from the foliage of *S. babylonica* and *S. fragilis* trees in the eastern Free State (Table 4.1). A total of 24 insect species were collected from the 40 *S. babylonica* trees (Figure 4.2), and 23 insect species from the 40 *S. fragilis* trees (Figure 4.3). The cumulative species curves indicated that sufficient sites had been sampled of *S. babylonica* but further sites needed to be sampled to get a good representation of the total diversity of insects that interact with *S. fragilis* (Figures 4.2 and 4.3). Herbivorous insect species were the most abundant across both tree species (74% of all sampled invertebrates), followed by predators (12%). Herbivores made up 80.6% of the total insects on *S. fragilis* and 65.6% on *S. babylonica* (Figure 4.4). Fourteen insect species were collected from both *S. babylonica* and *S. fragilis* (Figure 4.5), the most common species being the beetle *Plagiosterna discolor* (Stål) (subfamily Chrysomelinae) (Figure 4.6), the giant willow aphid *Tuberolachnus salignus* (Gmelin) (subfamily Lachninae), (Figure 4.7); the leafhopper *Helionidia quadrimaculata* (Naudé) (subfamily Typhlocybinae); and larvae of the sawfly *Nematus oligospilus* (Förster) (subfamily Tenthredinidae). *Helionidia quadrimaculata* leafhoppers and *P. discolor* beetles were the most frequently caught insects on *S. babylonica*, while *P. discolor* beetles and *N. oligospilus* sawfly larvae were frequently caught on *S. fragilis* trees.

Galls were observed on 27.5% of the *S. babylonica* trees sampled. In contrast, no galls were observed on *S. fragilis* trees. Galls were observed on roots, branches and trunks of *S. babylonica* trees (Figure 4.8).

Table 4.1: List of insects and arachnids found on *Salix babylonica* and *S. fragilis* trees over 13 sites in the eastern Free State, South Africa. Insects are organised in their orders and families, but those that were not identified to species level are labelled as morphospecies.

		Salix babylonica			***Salix fragilis***		
Order	**Invertebrate family/species**	**Insects per tree (Mean ± SE)**	**Infested Trees (%, N=40)**	**Total Number of Invertebrate Individuals**	**Insects per tree (Mean ± SE)**	**Infested Trees (%, N=40)**	**Total Number of Invertebrate Individuals**
	Insecta						
Coleoptera	**Anthicidae**						
	Anthicidae sp 1	0	0	0	1	2.5	1
Coleoptera	**Cerambycidae**						
	Cerambycidae sp 1	0	0	0	1	2.5	1
Coleoptera	**Chrysomelidae**						
	Plagiosterna discolor	2.5 ± 0.5	30	30	2.62 ± 0.37	52.5	55
	Chrysomelidae sp 1	1	2.5	1	1	5	2
	Chrysomelidae sp 2	1	2.5	1	0	0	0
	Chrysomelidae sp 3	1	2.5	1	0	0	0
	Chrysomelidae sp 4	1	5	2	2.33 ± 0.61	15	14
Coleoptera	**Coccinellidae**						
	Coccinellidae sp 1	1	5	2	1	5	2
	Coccinellidae sp 2	1.14 ± 0.14	17.5	8	1	5	2
Coleoptera	**Scarabaeidae**						

	Scarabaeidae sp 1	1	2.5	1	0	0	0
Coleoptera	**Tenebrionidae**						
	Tenebrionidae sp 1	0	0	0	1	2.5	1
Diptera	**Culicidae**						
	Culicidae sp 1	1.31 ± 0.13	32.5	17	1.75 ± 0.35	20	14
	Dryinidae						
	Dryinidae sp 1	1	10	4	0	0	0
Diptera	**Syrphidae**						
	Syrphidae sp 1	0	0	0	1	2.5	1
Diptera	**Tephritidae**						
	Tephritidae sp 1	0	0	0	1	2.5	1
Hemiptera	**Aphididae**						
	Tuberolachnus salignus	3 ± 2	5	6	3.22 ± 0.94	22.5	29
Hemiptera	**Pentatomidae**						
	Pentatomidae sp 1	0	0	0	1	2.5	1
	Pentatomidae sp 2	1	2.5	5	1	2.5	1
Hemiptera	**Psyllidae**						
	Helionidia quadrimaculata	1.82 ± 0.29	42.5	31	2.4 ± 0.87	12.5	12
	Psyllidae sp 2	0	0	0	1	2.5	1
Hymenoptera	**Braconidae**						

	Braconidae sp 1	0	0	0	1	2.5	1
	Chrysopidae sp 1	0	0	0	1	2.5	1
	Chrysopidae sp 2	1	5	2	0	0	0
Hymenoptera	**Formicidae**						
	Formicidae sp 1	1.09 ± 0.09	27.5	12	1.57 ± 0.57	17.5	11
	Ichneumonidae						
	Ichneumonidae sp 1	1	2.5	1	0	0	0
	Ichneumonidae sp 2	1	2.5	1	0	0	0
	Noctuidae						
	Noctuidae sp 1	1	2.5	1	0	0	0
Hymenoptera	**Tenthredinidae**						
	Nematus oligospilus	1	12.5	5	0	0	0
	Nematus oligospilus (larvae)	1.8 ± 0.41	25	18	2.83 ± 0.66	30	34
	Tenthredinidae sp 1	0	0	0	1	2.5	1
	Tenthredinidae sp 2	1	2.5	1	1	2.5	1
Lepidoptera	**Crambidae**						
	Crambidae sp 1	1	5	2	1	2.5	1
Neuroptera	**Chrysopidae**						
	Chrysopidae sp 1	0	0	0	1	2.5	1
	Chrysopidae sp 2	1	5	2	0	0	0

Psocoptera	**Psoidae**						
	Psoidae sp 1	1	20	8	1	5	2
	Acari						
	Orange mite	0	0	0	1	2.5	1

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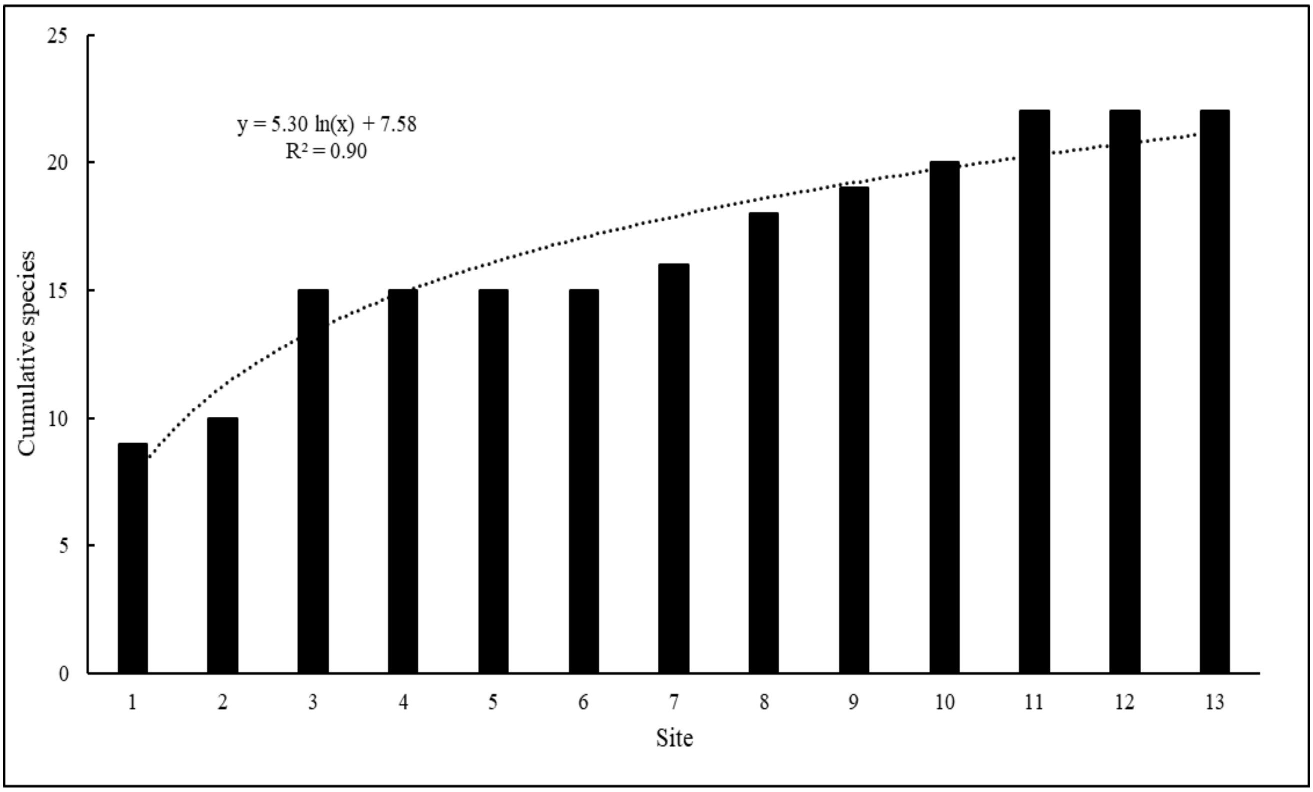

Figure 4.2: Cumulative number species of invertebrates interacting with *Salix babylonica* trees over thirteen sites in the eastern Free State, South Africa, analysed using regression.

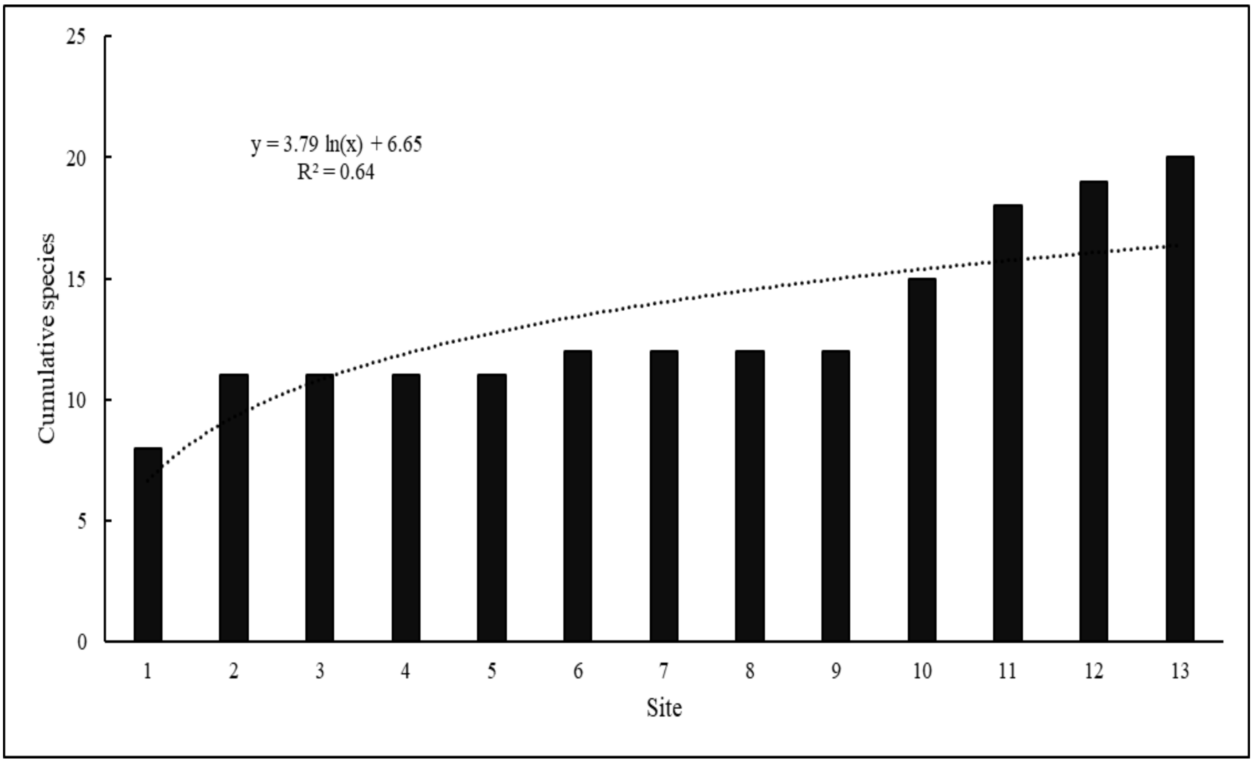

Figure 4.3: Cumulative number of species of invertebrates interacting with *Salix fragilis* trees over thirteen sites in the eastern Free State, South Africa, analysed using regression.

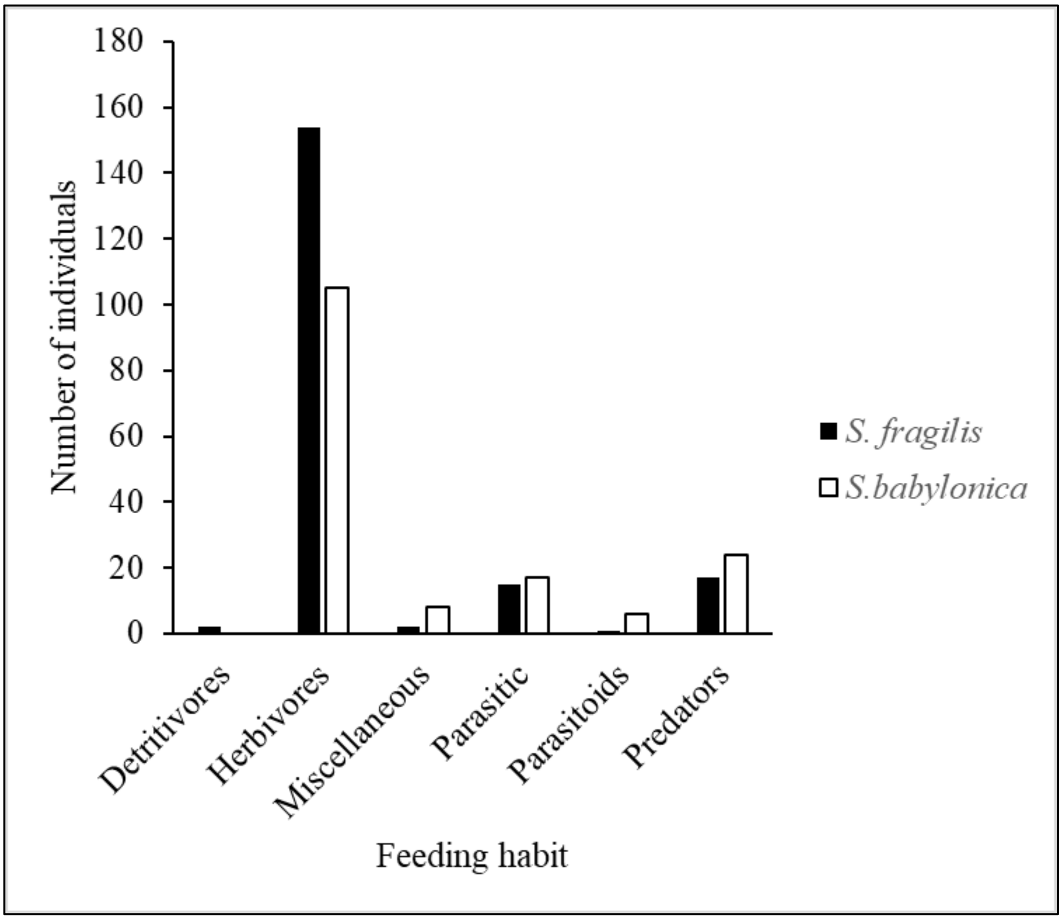

Figure 4.4: Feeding habits of insects and an arachnid found on *Salix babylonica* and *S. fragilis* tree foliage from thirteen sites in the eastern Free State, South Africa.

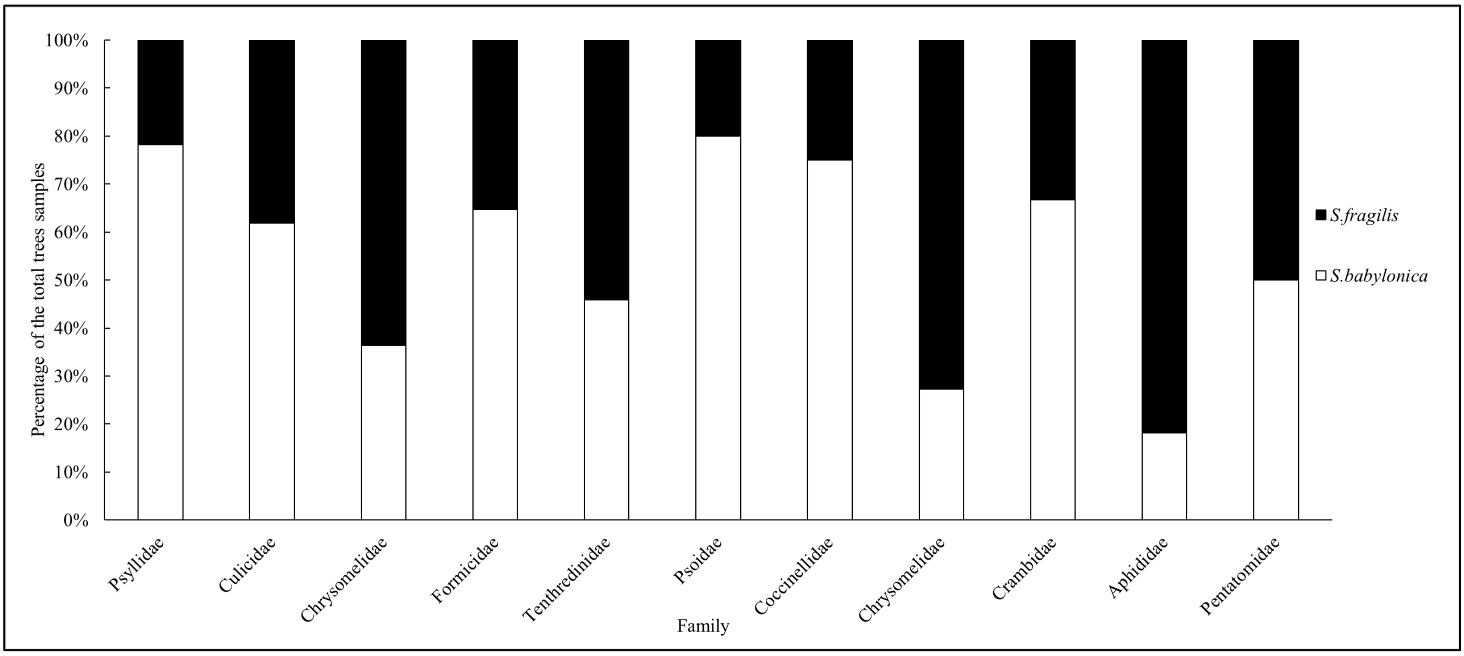

Figure 4.5: Species overlap between insect species within each family found on both *S. babylonica* and *S. fragilis* trees over 13 sites in the eastern Free State, South Africa.

Figure 4.6: Photograph of *Plagiosterna discolor* on *Salix fragilis* on one of the sites located in Phuthaditjhaba, Free State South Africa (Photograph by T. Mashamba).

Figure 4.7: Picture of *Tuberolachnus salignus* on *Salix babylonica* on one of the sites located in Phuthaditjhaba, Free State South Africa (Photograph by T. Mashamba)

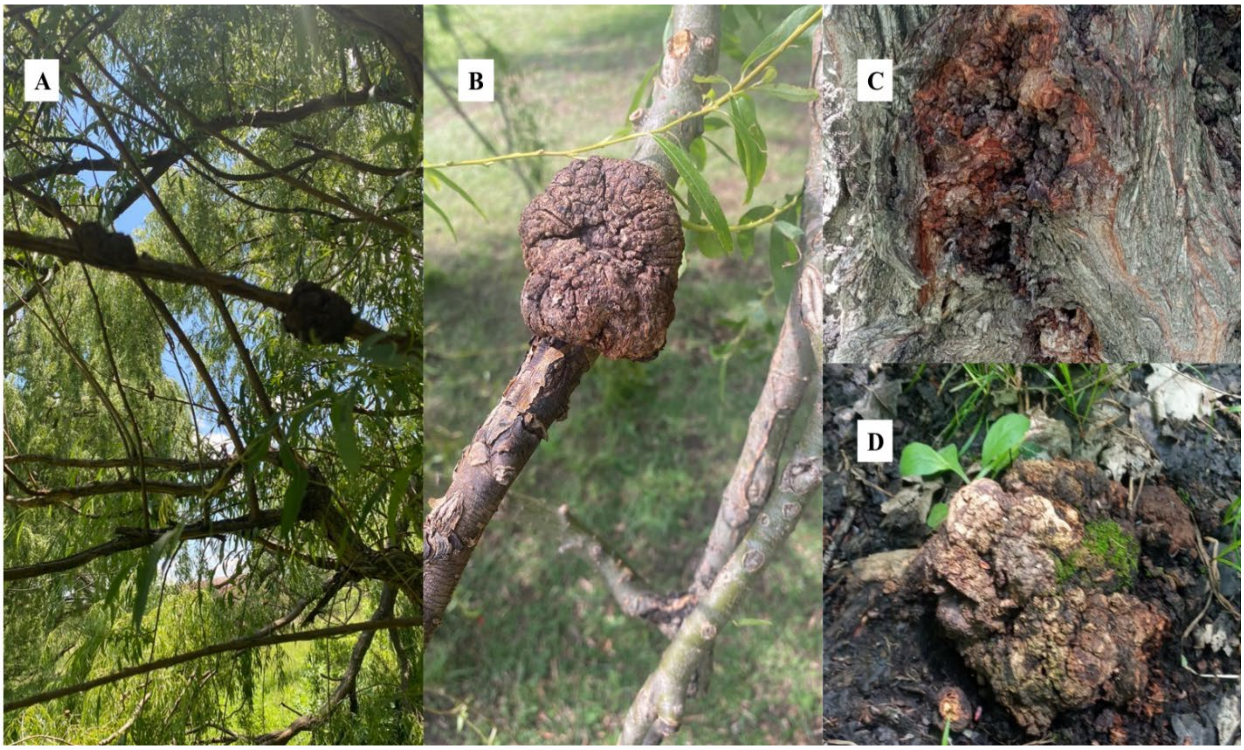

Figure 4.8: Galls observed on (A) branches, (B) branchlets, (C) trunk, and (D) roots of *Salix babylonica* in the eastern Free State, South Africa. (Photographs by T. Mashamba)

Discussion

This study documented invertebrates interacting with *S. babylonica* and *S. fragilis* trees from 13 sites within the grassland biome of the Eastern Free State, South Africa, yielding a total of 348 individuals representing 21 insect families, plus one arachnid. Among the collected insects, 14 species from 10 families were found on both *S. babylonica* and *S. fragilis*, indicating a shared assemblage of herbivores and predators across these willow species.

The first species of insect found on both willow species was *Plagiosterna discolor* (Chrysomelinae) (Figure 4.6). The larvae and adults of *P. discolor* have been observed feeding on the foliage of various willow species. A related species, *P. lutescens* (Thunberg), is known to feed on *Dovyalis caffra* (Hook.f. & Harv.) Warb. (Salicaceae). The indigenous host plant for *P. discolor* is *S. mucronata* Thunb. subsp. *mucronata*, based on unpublished data from a limited series of specimens in the South African National Collection of Insects (SANC). However, *P. discolor* appears to readily accept other *Salix* species, including *S. babylonica*, *S. fragilis*

(Agricultural Research Council, 2024), *S. matsudana* (non-indigenous), and *S. viminalis* (non-indigenous). The second observed species was *Tuberolachnus salignus* (Lachninae), the giant willow aphid (Figure 4.7), commonly found on *S. babylonica* and *Populus nigra*. Both winged (alate) and wingless (apterous) forms of this species exist. The giant willow aphid is commonly found on *S. babylonica* and *Populus nigra* L. (Barkat et al., 2021). In South Africa, *T. salignus* has been recorded in Ceres, Clarens, Elandshoogte, Springs, and Stellenbosch (Blackman and Eastop, 1994). The third observed species was *Helionidia quadrimaculata* (Typhlocybinae) (Naudé), a leafhopper from the subfamily Typhlocybinae. According to Theron's (1977) review of Naudé's specimens, two males and four females were collected from *S. babylonica* by E.S. Cogan at Cedara (KwaZulu-Natal) in 1917. Additional specimens were collected from Hammarsdale, Stellenbosch, and Swellendam. A closely related species, *Helionidia bomansi*, was described from Zaire (Dworakowska, 1981). Typhlocybinae leafhoppers, such as *H. quadrimaculata*, possess short mouthparts and feed on the palisade cells of plants rather than conductive tissue, which prevents them from acting as vectors of plant pathogens. However, their feeding can cause direct damage to the host plant, potentially allowing secondary pathogen infections (Theron, 1977; Dworakowska, 1981; Dworakowska, 1992). The last species of interest was *Nematus oligospilus* (Tenthredinidae) originally from Europe (De Tillesse et al., 2007), an invasive species in South Africa, inadvertently introduced to the Southern Hemisphere, including South Africa, New Zealand, and Australia, where it rapidly expanded its range (Caron et al., 2014). The prevalence of *N. oligospilus* on both willow species was first documented in the Highveld Region of South Africa and the Lesotho Highlands between 1993 and 1994 (Urban and Eardley, 1995). This study confirms a potential shift or expansion in its distribution or host range that warrants further investigation. *Nematus oligospilus* primarily affects *Salix* species, including *S. babylonica*, *S. fragilis*, and *S. humboldtiana* (Urban and Eardley, 1995; Malagon-Aldana et al., 2017), with minimal impact on other plants (Caron et al., 2011) or the native willow, *S. mucronata*. In South Africa, it primarily affects alien willow trees, causing severe defoliation (Urban and Eardley, 1995), which may have lasting effects on plant growth and regrowth, potentially impacting tree regeneration (Helbig et al., 2021). Successive defoliation by *N. oligospilus* results in drying out of the trees and eventually death.

These findings support existing literature, where *P. discolor* has been noted for feeding on willow foliage (Sprecher-Uebersax and Daccordi, 2016), and *T. salignus* is recognized as a common inhabitant of *S. babylonica* (Sopow et al., 2017) and a pest of *S. fragilis* (Tun et al., 2022) in their invaded ranges. The consistent presence of these species shows their ecological significance in willow habitats. Given the narrow range of *N. oligospilus* (Caron et al., 2011) this insect could be important in managing *S. babylonica* and *S. fragilis.*

Crown galls in woody plant species are often caused by an infection with the bacterial species, *Agrobacterium tumefaciens* (Klein and Link, 1955). The bacteria alter the infected part of the tree by inducing uncontrolled cell division, and production of auxin and cytokinin (Kerpen et al., 2019). This leads to the formation of tumorous growths, often at the crown of the plant where the stem or root join, but can occur on any plant part (Smith, 1916). Crown galls are prone to decay, making them susceptible to secondary infections (Smith, 1916). Crown gall disease has been observed in New Zealand affecting populations of poplars and willows (Spiers, 1980). It affected both the native species like *S. matsudana* and *S. purpurea* as well as non-native species resulting in the search for a biological control using non-pathogenic *Agrobacterium* strains to control crown gall disease in native *Salix* species, although complete control is challenging due to resistant pathogens (Spiers, 1980). Crown galls have been found to affect both ornamental and fruit trees. For example, in United States, it affects willows and poplars as well as susceptible fruit trees like apple trees, blueberries and grapes (Gauthier and

Embrey, 2018). These infections were found in a quarter of the *S. babylonica* trees sampled in this study and could be causing mortality of old trees, whether through secondary infections or by direct impacts. Since gall-forming bacteria are known from fruit trees in South Africa (e.g. apples; Mouton, 2023) and gall-forming bacteria have spread from invasive to native willows in New Zealand, the links between the bacteria infecting weeping willows and other woody species in South Africa need to be assessed to identify if the bacterium is a threat or just facilitating the management of the invasive willows.

The presence of 21 insect families and 14 shared species on both willow species suggests that *S. babylonica* and *S. fragilis* offer similar ecological niches for a variety of insects. However, the variation in insect abundance and species composition between the two willow species highlights the nuanced interactions between these plants and their associated herbivores. Leafhoppers and *P. discolor* were the most commonly collected insects on *S. babylonica*, while *P. discolor* and *N. oligospilus* were more frequently observed on *S. fragilis*. This distribution pattern may reflect differences in the plant species' susceptibility to specific herbivores or variations in plant chemistry that influence insect preferences. The single mite collected was identified as a predator rather than an herbivore, indicating a role in the ecosystem as a potential regulator of herbivore populations, though its specific impact was not assessed in this study.

Overall, this study contributes valuable insights into the insect communities associated with willows in the Eastern Free State. The novel finding of *N. oligospilus*' prevalence emphasizes the need for ongoing monitoring and further research to understand its ecological implications and management considerations of willows.

There are several considerations that could enhance future research into the invertebrates associated with invasive willows in South Africa. The reliance on sweep net sampling in this study may have skewed results toward more mobile and surface-dwelling insects, potentially missing less active or hidden species. To address this, future studies could incorporate a variety of sampling methods to capture a broader range of insect types, including those that are less mobile or more cryptic such as wood and stem borers, and gall-forming insects. Additionally, extending the study period to encompass different seasons or years would offer a more comprehensive view of long-term trends and seasonal variations in insect populations. Expanding the geographical scope to include other regions with *Salix* species would help determine if the observed patterns are consistent across different environments. Studies focusing on seasonal and yearly changes in insect populations could provide deeper insights into their temporal dynamics. Furthermore, investigating the influence of environmental factors on insect-plant interactions, such as climate change and habitat alteration, would contribute valuable information for ecosystem management and control strategies for *S. babylonica* and *S. fragilis*. Future research can also investigate gall formation in *S. babylonica* to identify the species causing gall formation in South Africa and if it is causing the observed decline of this invasive species. The specific bacterial species infecting and causing crown galls on weeping willows in South Africa is unknown and its identity and impacts need to be investigated further.

Conclusions

This study highlights the intricate associations between insects and *Salix* species within the Grassland biome of the Eastern Free State. By identifying key insect species and their interactions with willows, this research contributes to a better understanding of the ecological dynamics in this region and potential candidates for investigation as biocontrol agents.

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CHAPTER 5

GENERAL DISCUSSION

Introduction

The primary objective of this study was to evaluate the distribution, population dynamics, and invertebrates associated with *Salix babylonica* and *Salix fragilis* in the Grassland biome of South Africa. This was achieved by assessing historical and current distributions, population demography, and ecological interactions through aerial imagery, Google Street View (GSV) surveys, roadside surveys and field observations. The study also aimed to test the reliability of GSV as a tool for monitoring invasive alien plants (IAPs) and provide insights for effective management. This chapter serves to summarise the main findings of the study and discuss future research possibilities.

***Salix baylonica* and *Salix fragilis* as invasive species**

Salix babylonica and *S. fragilis* have invaded numerous parts of the world, with varied ecological impacts in different ecosystems. According to GBIF data, extensive invasions of *S. babylonica* have been observed in Australia (1,659 records), South Africa (608), New Zealand (132), Chile (101), Brazil (73) and Argentina (44). (Pauchard et al., 2020; Robinson et al., 2020; Zalba et al., 2021; Randall et al., 2023; Champion et al., 2024; GBIF, 2024). Fewer invasions have been recorded on the GBIF database for *S. fragilis* but with a similar distribution in these Southern Hemisphere countries (Australia: 1,327 records; South Africa: 38; New Zealand: 920; Argentina: 18; Chile: 4; Brazil: 1) (Pauchard et al., 2020; Robinson et al., 2020; Zalba et al., 2021; Randall et al., 2023; Champion et al., 2024; GBIF, 2024).

In suitable areas in Australia, willows dominate riparian vegetation. *Salix fragilis* and *S. x rubens* (basket willow) are by far the most widespread and abundant willows, and the biggest problem in the state of Tasmania, where they were introduced for erosion control along thousands of kilometers of streams in southeastern part of Australia (Baker, 2009). *Salix babylonica* is also regarded as invasive along the Lower River Murray, with *S. babylonica* occurring more commonly upstream and *S. fragilis* downstream (Kennedy et al., 2003). Within this system, both species show high water use, and their removal could potentially return water to the environment in drought-affected areas (Doody et al., 2014).

Similarly, in New Zealand, *S. fragilis* spreads exclusively along streams and is considered a nuisance weed (van Kraayenoord et al., 1995; Baker 2009). Here, some water resource managers are implementing willow removal and poisoning programs, but a conflict of interest exists as removing willows can degrade eel habitat whereas not removing the willows allows this invasive species to spread further (Meurk, 2011).

Very little has been done in South America about invasive trees including invasive *Salix*. However, two recent studies highlighted the growing presence of *S.* x *rubens* (a hybrid willow species) along watercourses at altitudes between 826 and 1648 meters above sea level in the highlands of southern Brazil (Sühs et al., 2020). Through field surveys, 1929 individuals of *S.* x *rubens* were identified, mostly near rivers and floodplains, with some populations spreading more than 100 meters away from plantations within 50 years (Sühs et al., 2020). The species likely spreads through water transport (Sühs et al., 2020). The study called for control measures and guidelines for safe cultivation to minimize environmental impacts in Brazil. The second study, in northern Patagonian streams, showed that *S. fragilis* alters aquatic invertebrate communities and slows leaf litter decomposition compared to native species, although its overall impact on small streams appears limited (Serra et al., 2012).

Despite the impacts known globally, the suggested impacts made by Henderson (1991), and the large populations found in South Africa, management against invasive *Salix* species in South Africa has not been implemented.

Management recommendations of *Salix* in South Africa

Management of Invasive Alien Plants (IAPs) in South Africa is crucial for profitable and sustainable crop production (Pieterse, 2010) as well as conservation of the country's ecosystem services (van Wilgen et al., 2008). Fortunately, a number of conservation organizations and government agencies, including the Department of Forestry, Fisheries and Environment, and the Department of Agriculture, aim to minimize the economic, environmental, and social impacts of weeds and alien invasive plants.

The primary mechanism for prioritizing and managing invasive species in South Africa is the NEMBA regulations (Wilson and Kumschick, 2024). Unfortunately, neither *Salix babylonica* nor *S. fragilis* are listed under the NEMBA regulations (Chapter 1), although they are listed under the CARA Act of 1983. However, these regulations have become outdated and are infrequently implemented.

Therefore, it is important to establish a process to revise the regulatory lists in a more transparent and science-informed manner (Wilson and Kumschick, 2024). This has recently been addressed in South Africa through the implementation of the Alien Species Risk Analysis Review Panel (ASRAP) to determine the risk posed by these species (Kumschick et al., 2020).

It is recommended that both *S. fragilis* and *S. babylonica* be reviewed by this ASRAP system to potentially promote them to the NEMBA lists. Due to its distribution and impact, *S. fragilis* should be considered for placement under Category 1b under NEMBA due to its known broad spreading distribution and impacts.

According to the regulations used by Department of Environmental Affairs (2015), based on NEMBA regulations, a 1b categorisation would mean that *S. fragilis* must be controlled and property owners must control this invasive species within their properties. If an Invasive Species Management Programme has been developed to control *S. fragilis*, a person must control this invasive species according to that programme. Authorised officials should be permitted to enter properties to monitor, assist with or implement the control of *S. fragilis.*

Based on investigations from this study, *S. babylonica* should not be considered a major threat in the Eastern Free State and thus the grassland biome, as it does not spread via seeds and does not seem to be prolifically reproducing vegetatively. However, it is uncertain how the species will be ranked in the ASRAP system. Further investigations should be conducted in other areas of South Africa to determine if similar trends are observed. For example, the Vaal River is known to host a significant number of *Salix* trees (Henderson, 1991) but was not surveyed in this study.

Salix babylonica trees are dioecious, and an important finding of the current study was the absence of male *S. babylonica* trees, and a subsequent lack of spread via seed (Chapter 2). Stokes and Cunningham (2006) also concluded that *S. babylonica* was not spreading through seed set in Australia, limited by the low frequency of both sexes occurring together. Though both sexes are present in Australia they do not flower at the same time, not allowing for successful fertilization (Stokes and Cunninham, 2006). Thus, it is imperative that no males of the species are introduced into South Africa and legislation prohibiting additional introductions of *S. babylonica* should remain.

The declining populations of *S. babylonica* and low recruitment offer potential opportunities for government and the general public. In general, government legislation has been prohibitive regarding the removal of alien trees. More and more species find themselves on prohibited lists. *Salix babylonica* was previously regarded as a premier horticultural tree providing excellent shade in South Africa, but its planting has been prohibited for several years due to perceived threats of invasiveness. Now that more information is available, it may become possible to promote the planting of *S. babylonica* in ornamental settings due to its low invasive potential in South Africa. This is not unheard of and in a biological control context, where a species is regarded as under complete control due to biological control efforts, the species may become delisted from NEMBA lists (Zachariades et al., 2017).

Due to the rapid vegetative spread of *S. fragilis*, it should be regarded as a 1b Invasive species under the NEMBA regulations. This would require the development of a management plan for the species, which should include biological control as a management option.

Biological control

Chapter 4 investigated the diversity and abundance of invertebrates associated with invasive willows in the eastern Free State. Field collections revealed a few herbivorous pests of the willow, including a sawfly (*Nematus oligospilus*), which can cause substantial damage to the tree (Urban and Eardley, 1995).

If biological control is to be considered for *Salix* species in South Africa, the surveys conducted here are important to show if any pests (potential agents) are already present in the introduced range of the target species and thus should be considered when surveying in the native range (Dudley et al., 2008). For instance, in South Africa, *Tetramesa romana* (Eurytomidae) a biological control agent for the invasive weed *Arundo donax* (L.) was already in South Africa prior to its formal initiation as a biological control program (Canavan et al., 2019). Similarly, the unintentional introduction of four phytophagous insects from the native range of *Robinia pseudoacacia* L. (Fabaceae) in the USA to its invasive range in Europe has significantly impacted its populations there (Cierjacks et al., 2013).

Due to the broad distribution of invasive willow species, South Africa can benefit from other countries working on the management of the species. For example, in Australia, several insects and pathogens are being evaluated as biological control agents for invasive willows (Adair et al., 2006). Key agents thus far other than the known willow sawfly *Nematus oligospilus*

(Chapter 4), include galling insects such as *Rhabdophaga saligna* Loew (Cecidomyiidae), which redirect plant resources to gall tissue, weakening the trees; and sap-sucking aphids like *Chaitophorus vitellinae* Schrank (Aphidoidea), which damage leaves and twigs. Additionally, seed and flower feeders like *Rhabdophaga heterobia* Loew (Cecidomyiidae), target reproductive structures to limit seed dispersal. These agents are being considered due to their potential to disrupt willow growth and reproduction while minimizing non-target impacts in Australia, where no native *Salix* species exist (Adair et al., 2006). This is similar to South Africa where we only have one native willow species which will need to be considered, *S. mucronata* Andersson (Salicaceae). Currently, the willow sawfly (*Nematus oligospilus*), through its host specificity testing, shows promise as a biological control agent in the country (Caron et al., 2011). Testing revealed that *N. oligospilus* feeds and reproduces exclusively on *Salix*, with only minimal feeding on *Betula pendula* Roth (silver birch) and *Populus nigra* L (poplar) (Caron et al., 2011). While it poses no threat to native plants tested, continued monitoring is essential to evaluate its host range and ecological effects. This agent has also been shown from field surveys to be specific to invasive willows in South Africa (Urban and Eardley, 1995). To be considered an official biological control agent, it would require further laboratory-based host specificity testing and the development of a release report.

Crown Gall

Crown galls are likely caused by the common and widespread *Agrobacterium tumefaciens* (synonymous with *Rhizobium radiobacter*; Graham, 1964; Chapter 4). Both *Salix* and *Populus* are known hosts for *A. tumefaciens* (Gauthier and Embrey, 2018). Some decline in the invasiveness of *S. babylonica* could be attributed to the presence of this bacterium in the eastern Free State. After infections, galls result in poor growth and gradual dieback due to increased sensitivity to environmental stresses (Gauthier and Embrey, 2018). However, the identity and impact of the bacteria was not confirmed during this study and should be the focus of future studies. In addition, given that crown galls have been identified as a threat to native *Salix* in New Zealand (Spiers, 1980), it would be advisable to investigate if other native *Salix* species are affected by this specific strain found in the eastern Free State.

Legislation and public awareness

Salix babylonica was widely and regularly planted across South Africa between the 1920-1960 (Jordaan, 2005). Some landowners suggested during the survey that they could remember their parents walking along stream banks planting willow branches every 20 m. This large-scale planting may have resulted in the perception that *S. babylonica* was more invasive than it really was in the 1970s and 1980s (Henderson, 1991). However, the large-scale planting of the species has slowed since the 1980s, primarily due to implemented legislation and increasing public awareness of the impacts of invasive species (Novoa et al., 2017). Investigations of the general public's knowledge of IAPs in Europe, South Africa, and South America showed that South Africans had significantly greater knowledge of IAPs and the regulations governing them in comparison to citizens of other countries (Gervazoni et al., 2023). Education and outreach initiatives are crucial in changing attitudes towards invasive species. By providing information about the negative impacts of invaders on the environment and the reasons for their management, communities can better understand the importance of control efforts (Byrne et al., 2020). This increased awareness can lead to greater community engagement and support for legislation and policies aimed at managing invasive species effectively.

The future of obtaining invasive species occurrence data

When determining the distribution of invasive species, one of the most important drivers of obtaining the data is costs and plant identification confirmations. Historically in South Africa, the invasive species occurrence records were collected by The SAPIA programme, which were conducted by a botanist conducting extensive road surveys (Henderson, 2007). While this method provides accurate identification, it is no longer financially feasible, which likely led to the termination of the programme in 2018. Here we suggest that Google Street View offers an affordable tool for government and conservation managers in determining and identifying invasive species distributions (Chapter 2). It has also been used in a number of other studies regarding this topic (Deus et al., 2016; Barone, 2021; Kotowska et al., 2021). However, its limitation in accurate species identifications always needs to be considered. However, the increasing availability of high-resolution satellite imagery and rapid development of tools available to analyse these images offers another valuable cost-effective opportunity to accurately obtain invasive species occurrence data. For example, in 2024 researchers from the Agricultural Research Council released an Invasive Alien Tree Classification Map for South Africa's Cape Floristic Region. The map was created as part of the NASA BioSCape Project which used Sentinel-2 satellite imagery and a Random Forest machine learning classifier to create a 10-meter resolution map of the Cape Flouristic Region (Rebello, 2024). They were able to classify six key invasive alien tree classes across the Cape Floristic Region with an overall accuracy of 93%. This is another cost-effective tool that is able to accurately determine species occurrences over large areas. Unfortunately, this technology again might be limited to tree species and not able to identify plants to species level. For example, all Pine species are lumped together.

Limitations of Study

Three main limitations were encountered in this study. While GSV has proven useful for assessing tree distributions, its accuracy is primarily limited to large woody species that are nearby to the road. As trees are further away from the road, distinguishing between medium-sized trees, small shrubs, and vines becomes challenging, particularly for *S. fragilis*, which is less conspicuous than *S. babylonica*. Additionally, GSV coverage in South Africa is generally restricted to tarred roads, further limiting its applicability for mapping populations in remote areas (Chapter 2). Roadside surveys, though effective in confirming species presence, have limited spatial coverage and cannot account for populations located away from major road networks. This limitation is particularly relevant for *S. fragilis*, which is often associated with riparian zones that may not be visible from roads (Chapter 2). Working with historical aerial photographs posed significant challenges, not only due to image quality but also because the process of downloading, georeferencing, and visually interpreting these images within a GIS package requires a high level of technical expertise. Distinguishing *S. fragilis* in aerial images was particularly difficult, necessitating extensive ground-truthing to confirm species identification.

General conclusion

The invasive dynamics of *S. babylonica* and *S. fragilis* differ significantly, with *S. fragilis* emerging as the more aggressive invader due to its recruitment potential and dense stands along watercourses. Although *S. babylonica* appears to be in decline, its historical impact and water consumption still make it ecologically significant. Google Street View has been shown to be a useful tool for monitoring *Salix* populations but requires methodological refinements for improved accuracy. The findings from this study furthermore underscore the need for targeted management strategies to mitigate the ecological impacts of these invasive species and protect South Africa's riparian ecosystems.

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