Critical factors affecting seed dispersal and germination of invasive alien *Rosa rubiginosa* in the eastern Free State

Patricia Masole

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UNIVERSITY OF THE FREE STATE
UNIVERSITEIT VAN DIE VRYSTAAT
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External Supervisor: Dr Grant Douglas Martin

Internal Supervisor: Prof. Sandy-Lynn Steenhuisen

Co-Supervisor: Dr Stephanie Louise Payne

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Declaration

I, Patricia Masole do hereby declare that the report hereby submitted in fulfilment of the degree of requirements for a degree of Master of Science in Botany, in the Faculty of Natural and Agricultural Sciences, Department of Plant Sciences, University of the Free State (Qwaqwa campus) represents my own original, independent work that has not been submitted before for any degree or examination in any other university, and that all the sources I have used or quoted have been indicated and acknowledged as complete references.

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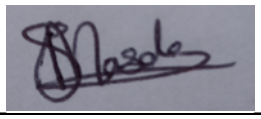
Signed 

Patricia Masole

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Abstract

Rosa rubiginosa L. (Rosaceae) is a common woody plant invader in the grassland biome of South Africa. It is originally from Eurasia and is classified as a category 1b invader in South Africa under the National Environmental Management: Biodiversity Act, 2004. This act prohibits the planting or harvesting of this species in South Africa. However, the impacts of *R. rubiginosa* on the environment and economy are complex, with both positive and negative effects that warrant further investigation. The objectives of this study were to investigate (1) various aspects of the reproductive ecology of *R. rubiginosa*, including fruit and seed production, the abundance and viability/longevity of seeds in the soil seed bank, and the importance of cold stratification for seed germination; (2) the impact of the seed predator *Megastigmus aculeatus* Swederus (Hymenoptera: Torymidae) on *R. rubiginosa* seeds; and (3) the potential for *R. rubiginosa* to facilitate woody invasions into the grassland biome of South Africa.

This study showed that for invading populations of *R. rubiginosa* on farms outside Clarens in the Free State of South Africa, seed germination success is low. An overall percentage of 1.25% of seeds exposed to cold stratification were observed to germinate within 24 months, while no seeds germinated from the control treatment (seeds stored at ambient temperature for the same amount of time) or from seeds collected every two weeks from the field. Seeds buried in material bags and having been exposed to soil ambient temperature in the soil, however, showed high levels of viability (90%), decreasing to only 83% after being buried in the soil for 17 months. Significantly more seeds were found in the soil under the *R. rubiginosa* canopy, compared to 2 and 8 m away from the parent shrub. The maximum number of fruits produced per shrub was 1 950 fruits on a shrub with a volume of 78 m^3, while the minimum number of fruits produced was 118 fruits by a shrub with a volume of 5 m^3. On average, *R. rubiginosa* shrubs in the studied populations had an average (± standard error) volume of 25 ± 3 m^3,

produced an average of 480 ± 57 fruits per shrub, and fruits produced an average of 18 seeds per fruit.

The potential of *Megastigmus aculeatus* to limit the spread of *R. rubiginosa*, and the proportion of seeds predated by the wasp was assessed. One hundred rosehips were randomly collected every month from March until September 2021. Only a total of 148 *M. aculeatus* emerged from some of the 700 *R. rubiginosa* fruits, giving an overall percentage of 1.28% of seeds predated. This showed that the seed predator *M. aculeatus* does not have a large impact on the number of seeds entering the environment, produced by *R. rubiginosa* shrubs.

Differences in woody plant (> 10 cm height) diversity and abundance were assessed under three treatments: *R. rubiginosa* canopy, *Leucosidea sericea* Eckl. & Zeyh. (native) canopy, and open plots of herbaceous grassland (control), to assess if *R. rubiginosa* acts as a nurse plant and facilitates the recruitment of woody plant species. This study showed that *R. rubiginosa* had a more significant nurse plant effect compared to both *L. sericea* and open grassland (control), facilitating the establishment of both native and invasive species. There were significantly more native species than invasive species under all three treatments. Furthermore, more invasive species (13 species) were recorded growing under *R. rubiginosa* compared to both *L. sericea* (11 species) and in the control plots (11 species). Shrub size also affected the number of plant species recorded underneath each focal species with larger shrubs having more woody plant species underneath.

To investigate if *R. rubiginosa* is creating a different microclimate under its canopy, two temperature and light loggers were placed under *R. rubiginosa* shrubs and in open grassland. This showed that plants under *R. rubiginosa* experienced both lower average temperatures and light intensity (14.6 °C, 3940.5 lux respectively) while open grassland sites experienced both

a higher average temperature and more light (17.7 °C, 17544.36 lux respectively). Thus *R. rubiginosa* created a different microclimate compared to open grassland.

This study provides valuable insights into the reproductive ecology of *R. rubiginosa,* a species that has significant impacts on the environment and economy in South Africa. This study showed that cold stratification is needed for *R. rubiginosa* seeds to germinate and *M. aculeatus* has a poor limiting potential on *R. rubiginosa*. Furthermore, *R. rubiginosa* shrubs have been shown to produce many fruits, each of which have many seeds and these seeds can stay viable in the soil for a long time. *Rosa rubiginosa* is demonstrated to act as a nurse plant to both native and invasive species when compared to both native *L. sericea* and control sites, since it accommodates more seedlings of native species than invasive species when compared to both treatments. However, more research is needed to fully understand the complex relationships between *R. rubiginosa* and other plant species and investigate more effective potential biocontrol agents to further slow the spread of *R. rubiginosa* in South Africa. The experiments conducted here will add to the current knowledge of *R. rubiginosa* here in South Africa, aiding in determining the stage that is most feasible for their management.

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Chapter 1

1. General introduction

1.1. Invasive alien species

Alien species are known as exotic, foreign, non-indigenous, or non-native animals, plants and microbes that occur outside their natural range and have dispersal potential (IUCN, 2000). However, not all alien species are initially invasive, but many may become invasive with time as they spread uncontrollably and become fully established (Richardson et al., 2000). When an alien species becomes widespread, overly abundant, and starts having some form of impact then it is usually regarded as an invasive alien species ("IAS"; Richardson et al., 2000; Richardson and Pysek, 2010). These species usually have impacts on biodiversity by often decreasing the number of native species through competition or altered environmental conditions (Perrings et al., 2000; McNeely et al., 2001). In some cases, human enterprises, and critical resources such as trade, crop and livestock production, pastureland, forests, and natural resources; as well as human and animal health may be affected negatively by IAS (Mack et al., 2000, Mooney et al., 2005; Chikowore et al., 2021a). Invasive alien species are also responsible for substantial losses of goods, services, and production capacity (such as damaging infrastructure and altering ecosystem services; Bradshaw et al., 2016; Chikowore et al., 2021b). Furthermore, there are direct economic impacts of resources that are spent each year on their management (Hoffmann and Broadhurst, 2016).

In a recent publication, Diagne et al. (2021) suggest that IAS globally has cost at least US$1.288 trillion (2017 US dollars) over the past few decades (1970 - 2017), with an annual mean cost of US$26.8 billion. In addition, they estimated that the annual mean cost of IAS could reach US$162.7 billion in 2017, and does not show any sign of slowing down, exhibiting a consistent threefold increase per decade (Diagne et al., 2021). In South Africa, the current

ecological cost for both animal and plant invasive species is estimated to be more than R6.5 billion each year (South African Government News Agency, 2021).

Globally, new technologies have made the movement of humans and commodities easy and these movements facilitate the spread of IAS, both intentionally and unintentionally (Meyerson and Mooney, 2007; Blackburn et al., 2011). South Africa (SA) is one of the most invaded countries in the world, with both natural and semi-natural ecosystems seriously affected by alien species (Moran et al., 2013; Faulkner et al., 2016; Van Wilgen et al., 2020). The first records of intentionally introduced alien species into SA were in 1652 when Dutch horticulturalist Hendrick Boom started a garden in Cape Town in Western Cape Province for the Dutch East India Company (Pooley, 2009). In SA, a total of 107 alien species are suspected to have major negative impacts on biodiversity, and most (75%) of these are plants (Van Wilgen et al., 2018). Common IAS include *Anas platyrhinchos* L. (Mallards; Anatidae, Animalia), *Acacia saligna* (Labill.) H.Wendl (Port Jackson Willow; Fabaceae, Plantae) and *Carassius auratus* L. (goldfish; Cyprinidae, Animalia) (Smit et al., 2017; Van Wilgen et al., 2022). IAS consist of organisms from all six kingdoms of life, with IAS from the plant kingdom being considered the most damaging, attributed with the greatest environmental and economic impacts (McGeoch et al., 2010).

1.2. Invasive alien plants

Invasive alien plants (IAPs) are plants that are dispersed outside their native region either intentionally or unintentionally and can be harmful to the environment (Vilà et al., 2010). These plants usually grow faster, reach maturity more quickly, and produce more propagules while capitalizing more on additional nutrients compared to native plant species (Pyšek and Richardson, 2007; Van Kleunen et al., 2010; Thompson and Davis, 2011; Dawson et al., 2012; Rejmánek et al., 2013; Ratnayake, 2014). As a result, IAPs often flourish in both disturbed and

natural landscapes (Pratt et al., 2017). Global trade and travel continue to contribute to both intentional and accidental introductions of IAPs (Sala et al., 2000; Keane and Crawley, 2002; Richardson et al., 2003; McKinney, 2006; Faulkner et al., 2016), and the global distribution of plants recently showed a greater degree of international trade, with a majority of the trade involving IAPs (Moran et al., 2013; Van Wilgen et al., 2020). It is predicted that more IAPs are likely to reach SA due to its high number of international ports and often inadequate border regulation on imported goods (Mgidi et al., 2007; Faulkner et al., 2016).

1.3. Invasive alien plants in South Africa

Invasive alien plants have been a problem in SA for many decades which has resulted in a long history of research on plant invasions (Van Wilgen et al., 2020). According to Van Wilgen et al. (2020), there is no current, comprehensive inventory of alien, naturalized, and invasive plants in SA, and while 327 plant taxa, most of which are invasive, are listed in national legislation, several publications estimate that there are between 8 750 and 9 000 alien plant taxa in SA (Figure 1.1). Individual researchers started collecting distribution records in 1979, and by 2016 the Southern African Plant Invaders Atlas (SAPIA) database contained 87,000 records for 773 IAPs (Henderson and Wilson, 2017). Over half of these naturalized taxa are trees or shrubs; just under a tenth are in the families of Fabaceae (73 taxa) and Asteraceae (64 taxa); and genera with the most species are *Eucalyptus*, *Acacia*, and *Opuntia* (Richardson et al., 2020).

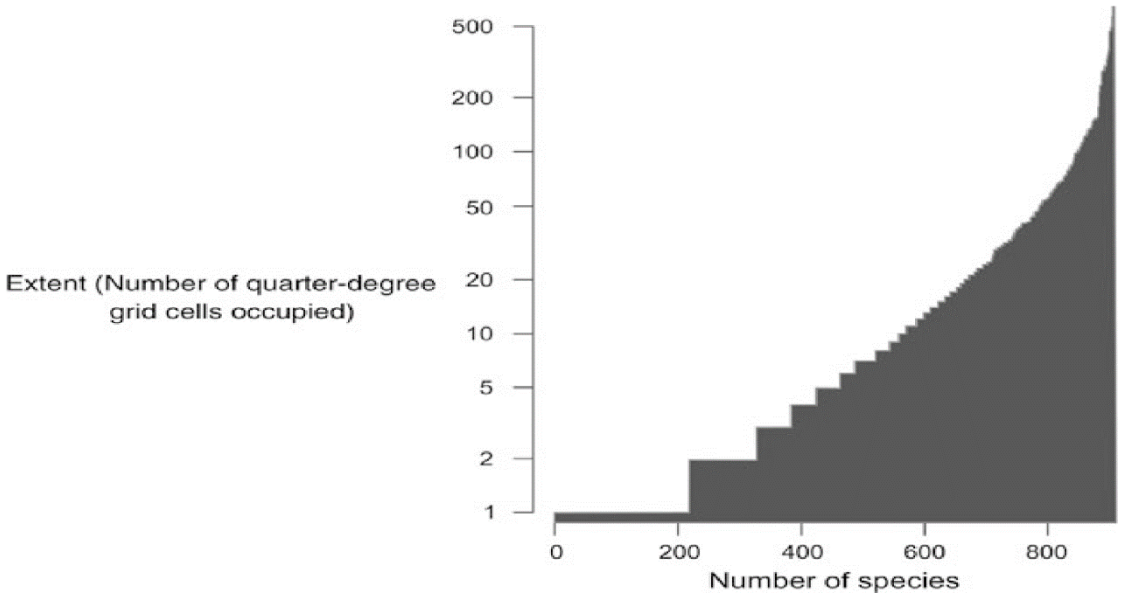

Figure 1.1: The broad-scale distribution of alien plants in South Africa as per the southern African Plant Invaders Atlas (SAPIA) The extent is measured as the occupancy of quarter-degree grid cells out of a total of 1804 cells (extracted from Van Wilgen et al., 2020).

There is a uniform assemblage of IAPs in SA, although Richardson et al. (2020) have categorised them into four ecologically distinct clusters, namely moist subtropical invaders, fynbos-specific invaders, semi-arid invaders, and grassland-specific invaders. These IAPs occur in high frequencies across multiple zones, mainly in riparian zones and other azonal habitats and some depend on human-mediated disturbance, which weakens factors that determine specificity to any biogeographical region (Richardson et al., 2020). However, many are restricted to the suggested zones, for example, species-rich fynbos shrublands are highly vulnerable to fire-prone species from across the world (Richardson et al., 2020). Furthermore, serotinous *Pinus* species from Europe and Central and North America, and Australian *Acacia* species with soil-stored seeds that are stimulated to germinate by fire, have invaded vast areas of fynbos and grasslands, transforming shrubland and grassland vegetation into woodlands or forests over several decades (Richardson and Kluge, 2008; O'Connor and Van Wilgen, 2020).

In the grassland biome, the cold winters also drive the invasion of species from the more northern temperature regions of the globe that are cold tolerant (Martin, 2021).

1.4. Invasive species in grasslands of South Africa

The grassland biome covers approximately 27% of SA (Eckhardt et al., 1993) and harbours a rich species diversity (Reyers and Tosh, 2003). The biome has the third-highest number of IAPs of the nine biomes, harbouring well over 320 species (Henderson, 2007). In addition, the grassland biome is one of the most poorly protected biomes in southern Africa as 23% is under cultivation, 60% is irreversibly transformed, while only 2% is protected, and most of the remaining natural area are used by livestock (Fairbanks et al., 2000). The invasion of this biome has negative impacts such as reduction in grass production benefiting the growth of invasive alien species (Chikowore et al., 2022). This, in turn, results in a negative impact on ecological services (e.g., grazing, surface water runoff, groundwater recharge, livestock production, and sustaining biodiversity; Van Wilgen et al., 2008; O'Connor and Van Wilgen, 2020; Chikowore et al., 2022). Additionally, invasive species can displace local species and transform the vegetation structure (O'Connor and Van Wilgen, 2020). This change in vegetation structure can result in changes in the hydrology of watercourses and reduce stream flow (Le Maitre et al., 2020; Richardson et al., 2020). Furthermore, IAPs are known to cause a reduction in conservation and tourism value, increase soil erosion and the consequent siltation of dams and rivers, changes in the natural soil composition, and oxygen depletion in water (Bromilow, 2010; Westwood, 2020). The dominant IAPs in the grassland biome include woody species from the genera *Acacia*, *Eucalyptus*, *Pinus*, and *Populus* (Richardson et al., 2020). In addition to these common IAPs, a new suite of invasive Rosaceae species has been recorded in the grassland biome (Canavan et al., 2022). One of the most frequent and prominent invaders from

the Rosaceae family is *Rosa rubiginosa* L. (Henderson, 1991; Carbutt, 2012; Canavan et al., 2022).

1.5. *Rosa rubiginosa*

1.5.1. Species description

There are approximately 100 species of *Rosa* which are believed to have arisen by hybridisation, often accompanied by polyploidization, either naturally or during cultivation, many of which are morphologically similar (Smulders et al., 2011; De Riek et al., 2013, Zhang et al., 2013). In addition, environmental conditions can create morphological variations in species (Lewis, 1957). There are several *Rosa* species introduced in SA, such as *Rosa multiflora* Thumb., *Rosa* x *odorata* (Andrews) Sweet., and *R. rubiginosa* (Westwoood, 2020).

Rosa rubiginosa (Rosaceae), also known as *R. eglanteria*, and *R. mosqueta* is commonly known as sweet briar or eglantine rose (Täckholm, 1920). It is native to Europe and Asia (Eurasia) and has become naturalized in North America, South America, Australasia, and Africa (Henderson, 2007; Hirsch et al., 2011). *Rosa rubiginosa* is a dense deciduous shrub, approxiately 2 - 3 meters high and across, growing in habitats exposed to full sunlight but can grow in partial shade (Aguirre et al., 2009; Gadzinowska et al., 2019; Figure 1.2a and 1.2b). The stems are erect and heavily armed with stout, hooked thorns and prickles mixed with scattered aciculi and setae (Molloy, 1964). It has leaves that are bright green with 5 - 7 leaflets each (Aguirre et al., 2009). Leaflets are ovate or obovate to approximately circular, 10 - 25 mm long, 10 - 15 mm wide, and with toothed margins (Aguirre et al., 2009). *Rosa rubiginosa* bears small clusters of brightly coloured (mostly pink) flowers (20 - 40 mm) with glandular-hairy peduncles with odours that attract insects (Aguirre et al., 2009; Cavallero and Raffaele, 2010). The fruits (rosehips) are orange-red, ovoid to globose, 15 - 20 mm long, and with prickles (Aguirre et al., 2009).

This highly invasive shrub prefers habitats such as moist valleys, rocky outcrops, watercourses, roadsides, as well as overgrazed land (Henderson, 2001). Its ability to invade regions may be due to it being a self-fertile, apomictic, clonal species that is able to reproduce sexually (Zimmermann et al., 2010).

(a) (b)

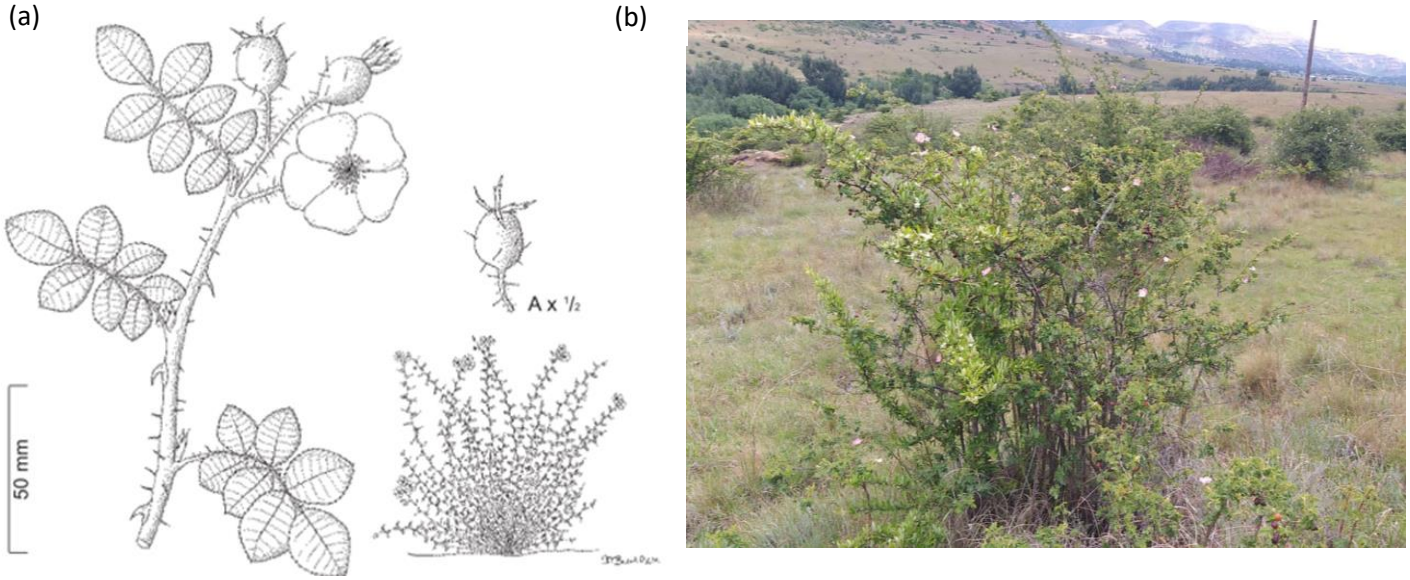

Figure 1.2: (a) Line drawing of *Rosa rubiginosa* leaves, flowers, rosehips, and shrub. Drawn by Sandie Burrows, first published in Henderson (2001). (b) *R. rubiginosa* shrub with leaves, flowers and rosehips observed in Clarens, eastern Free State Province, South Africa.

1.5.2. *Rosa rubiginosa* in South Africa

The National Environmental Management: Biodiversity Act (NEM:BA, 2004), categorises *R. rubiginosa* as a 1b invader indicating that planting or harvesting of this plant is prohibited in SA (AIS, Republic of South Africa, 2014; Figure 1.3). It was also recorded as a troublesome weed and included in a list of 17 exotic weed species under consideration for biological control (extracted from Weber, 2017). In southern Africa, *R. rubiginosa* primarily invades the grassland biome and high elevation of SA and Lesotho where it can form dense stands (Martin, 2021).

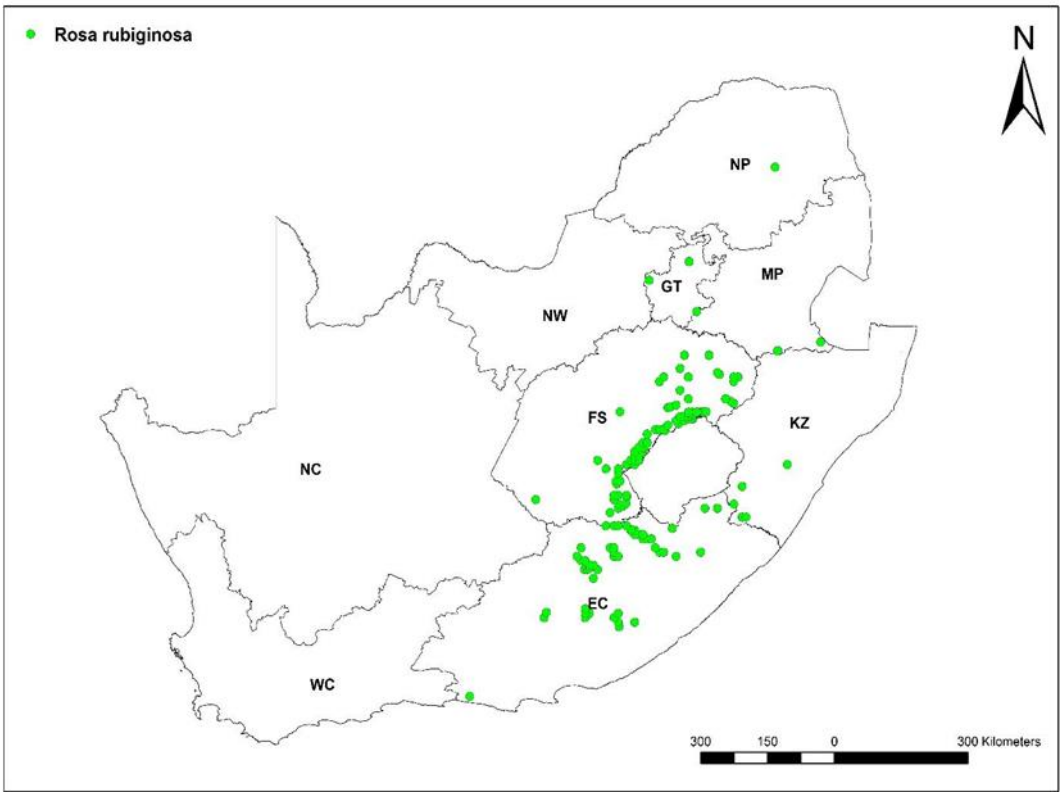

Figure 1.3: Distribution records of *Rosa rubiginosa* L. in South Africa. Provinces: Gauteng (GP), Mpumalanga (MP), Limpopo (LP), North West (NW), KwaZulu-Natal (KZN), Eastern Cape (EC), Western Cape (WC), Northern Cape (NC) and Free State (FS) (Westwood, 2020).

1.5.3. Negative impacts of *Rosa rubiginosa*

In general, there have been limited studies on the impacts of *R. rubiginosa* (Westwood, 2020). However, the high seed production in conjunction with rapid growth rates allows this shrub to rapidly invade open areas forming dense monospecific stands (Zimmermann et al., 2011; Carbutt, 2012). Furthermore, Westwood (2020), who interviewed a series of invasion biologists about their opinions of the species as an invasive in SA, concluded that *R. rubiginosa* has a negative impact through three key processes: creating impenetrable thickets, reducing cultivated land, and reducing grazing land. In addition, *R. rubiginosa* impacts water availability and it is estimated that the total area in SA that is invaded by *R. rubiginosa* is 11 801 ha, resulting in a reduction of an estimated 8,75 million m^3 of mean annual surface water runoff,

which is the equivalent of 74 mm of rainfall (Le Maitre et al., 2016; Van Wilgen et al., 2018). In addition, since this shrub produces thickets, it blocks livestock from accessing water sources (Grundy, 1989).

Svriz et al. (2013) in Argentina states that *R. rubiginosa* facilitates the regeneration of both invasive and native species found underneath their canopy and interestingly it can invade the disturbed area. In addition, *R. rubiginosa* is highly effective in being used for soil reclamation, is resistant to environmental stresses, and can grow on salt-contaminated urban soils along transport routes (Williams, 1997). However, *R. rubiginosa* is an alien species in these systems and could have negative ecological impacts by competitively excluding indigenous species (Gadzinowska et al., 2019).

1.5.4. Conflict of interest species

In addition to restoration uses, *R. rubiginosa* positively contributes to the sustainability of various rural communities' economies and can be regarded as a conflict species (Aguirre et al., 2009; Zimmermann et al., 2010; Vermaak et al., 2011). *Rosa rubiginosa* fruits (rosehip fruits) have been used over the years to make various herbal teas and jams (Smulders et al., 2011) and are actively collected and sold by local people in Lesotho (Van der Walt et al., 2016) and parts of SA. Rosehips products are in the cosmetic industry, increasing the market demand for rosehips (Westwood, 2020). Seeds from rosehips contain up to 8% oil comprised of oleic, linoleic, linolenic, and *trans*-retinoic acids (Moure et al., 2001). This seed oil is used to accelerate the regeneration and healing of damaged tissues due to antioxidant activity (Moure et al., 2001). Rosehips are collected from naturalised populations as the species is not currently cultivated in Lesotho and cultivation is prohibited by law in SA (NEM: BA, 2004). According to Westwood (2020), one rosehip company could grow from 1 500 tons of raw rosehip to 20

000 tons if cultivation was allowed and that would result in an industrial growth turnover from R57 million to R760 million.

1.6. Problem statement

Most literature about *R. rubiginosa* derives from Europe, North America, and South America (Zlesak, 2005; Hirsch et al., 2011; Zimmermann, 2012; Svriz et al., 2013), while very little is from southern Africa. This means that the available literature, despite being on the same species, is often not suitable and transferable to the southern African or grassland biome context. For example, much of the literature from South America regarding *R. rubiginosa* is from savanna and forest systems and thus not appropriate for the grassland biome (Zimmermann et al. 2012), while literature from the USA for example by Porter (2022) suggests chemical management options as the best methods for management. However, no herbicide is currently registered for the control of *R. rubiginosa* in SA.

There has been some literature on *R. rubiginosa* from Lesotho and SA. In South Africa, Westwood (2020) discussed the landowner and stakeholder perceptions of the *R. rubiginosa* invasion, while Canavan et al. (2022) explored several Rosaceae species, including *R. rubiginosa*, that are increasingly invading mountainous regions of SA. Mokhobo (2018) and Rahlao et al. (2023) investigated some aspects of *R. rubiginosa* such as seed ecology and the ecological impacts of the species on rangelands in Lesotho. However, there is still limited information on *R. rubiginosa* seed ecology in the field. *Rosa rubiginosa* is known to have delayed germination and it can take up to two years for the seeds to germinate (Huxley, 1992). Damascos et al. (2004) in Argentina showed that *R. rubiginosa* seeds vary between 18 473 seeds/m^2 in forest areas and 22 060 seeds/m^2 in the steppe. The current study aimed to answer questions that have not been researched in SA such as an estimation of seeds and/or fruits a *R. rubiginosa* shrub produces and number of seeds each rosehip fruit contains. While Southern

African literature on *R. rubiginosa* is limited, there is recent research on the rapid spread of other invasive Rosaceae such as *Pyracantha angustifolia* (Adams et al., 2023) and *Cotoneaster pannosus* (Moloi, 2022) in the grassland Biome (see also Canavan et al., 2022). These studies investigated how seed ecology of these other invasive Rosaceae species contributed to their success in the grassland biome, and it is thought that seed ecology may play an important role in the spread and persistence of this family. This study focused on the seed ecology of *R. rubiginosa*, and how this species facilitates the spread of other species in grassland, which has not been investigated in SA. Furthermore, in SA, the shrub has acquired a non-native seed parasitoid *Megastigmus aculeatus* var. *nigroflavus* Hoffmeyer, and its potential to reducing the spread of *R. rubiginosa* seeds was studied..

1.7. Aims and objectives

This study aimed to investigate various aspects of the reproductive ecology of the invasive alien species *R. rubiginosa* and its facilitation of spread of other woody encroaching species in the eastern Free State Province of SA. The results of this study will help inform management decisions of this conflict species when deliberating the benefits or negative impacts for local communities and the natural ecosystems it has invaded. In order to achieve the aims of this study, the following objectives were fulfilled: (1) determine the importance of cold stratification to *R. rubiginosa* seeds to germinate, (2) investigate the seed ecology of *R. rubiginosa* to determine their seed load, (3) determine the impact which the seed predator *M. aculeatus* has on *R. rubiginosa* seeds, and (4) determine whether *R. rubiginosa* facilitates woody invasions into the grassland biome in SA.

1.8. Dissertation structure

The structure of the following dissertation arranged in four chapters with the two data chapters prepared with the intention of publication. Chapter 1 presents a general introduction and Chapter 4 presents a general discussion and conclusion. Chapters 2 and 3 resemble the format of manuscripts with the aim of publication. All the experiments used *Rosa rubiginosa* as a focal study species. The first chapter introduces concepts, study species, and literature review on topics presented in the dissertation. The second chapter investigates the reproductive biology of this species including studies on germination trials, seed viability/longevity, soil seed bank, fruit and seed production, and quantifying seed predation in *R. rubiginosa*. The third chapter examines the recruitment of native and invasive under the canopy of this focal species. The last chapter provides a general discussion to link each experimental chapter, recommendations for management options, and improvements on the current study.

Chapter 2

Reproductive ecology of *Rosa rubiginosa* in the eastern great escarpment of South Africa

2.1. Introduction

2.1.1. Reproductive ecology in plants

Reproduction is a crucial step in the lifespan of plants that ensures species preservation (Tandon et al., 2020). Plant reproduction can either be sexual or asexual (e.g., vegetative), and the diversity between the two reproductive modes may vary widely between and within species (Zhang and Zhang, 2007). During sexual reproduction, fertile pollen from flowering plants is essential for fertilizing the central nucleus of an ovule and ensuring the production of viable and genetically variable seeds (Boldrini et al., 2006). After seeds mature, they may experience potential long-distance dispersal, delayed germination from a persistent seed bank, and genetic adaptation to new ecological conditions (Krebs, 1994). These characteristics can help a plant become dominant when introduced into a new environment (Tirado and Pugnaire, 2003; Burns et al., 2011).

Studies investigating seed ecology in invasion science are common due to the importance of seeds in plant population growth, spread, and persistence (Bond and Midgley, 2001). Seed dispersal studies showed that seed density decreases with increasing distance from the maternal species (Muller-Landau et al., 2008; Moloi, 2022; Adams et al., 2023). Many studies have demonstrated that a significant driver of invasion success lies in the seed and seedling stages because, without seeds and seedling survival, there may be no recruitment if the species cannot vegetatively propagate (Lambers and Clark, 2003; McAlpine and Jesson, 2008; Hooper and Dukes, 2010). Seedlings can either be vigorous or weak; these differences may be due to seed health and energy reserves which may be high or low (Howe and Richter, 1982). Seed size (i.e. large or small) and energy reserves within seeds can cause animal dispersers to differ (Howe

and Richter, 1982). The seed size also affects the plant life cycle since it is a critical trait often linked to plant fitness (Larios et al., 2014). Maximum fitness would be achieved by an individual plant that produces an infinitely large number of big seeds (Primack, 1987). This could be due to the evolutionary dynamics of seed size: seedlings from large seeds are better competitors and have a higher precompetitive survival rate than seedlings from small seeds (Geritz et al., 1999). Large seeds are typically found in forests where light is limited, allowing seedlings to be competitive in light-limited environments; while tiny seeds with rapid germination are usually found in open spaces, such as grasslands (Snow, 1971).

Various studies have demonstrated that invasive species have a reproductive advantage over native species, although these advantages may depend on the individual species' growing conditions (Goergen and Daehler, 2001; Vila and Weiner, 2004; Zheng et al., 2015). For example, some non-native invasive plants allocate a high proportion of resources to sexual reproduction but may also possess reproductive traits such as the ability to reproduce asexually or through apomixis, which may facilitate their success (Sun et al., 2018). However, the persistence of invasive alien plants (IAPs) often depends on high seed numbers, seeds that are viable for long periods in the seed bank, and are widely dispersed (Duarte et al., 2023).

2.1.2. Factors that influence seed survival and germination

Seed germination and seedling growth can be advanced or delayed by changes in soil temperature and burial depths (Teraminami et al., 2013; Zhu et al., 2014; Tao et al., 2022). The rate of seedling emergence may also be influenced by the amount of moisture in the soil at different burial depths (Ye et al., 2019; Tao et al., 2022). However, this only applies when seeds are sensitive to environmental conditions, such as temperature, light, storage periods (after-ripening), soil moisture content, and nitrate levels (Duermeyer et al., 2018). The size and number of seeds produced by invasive plants play a role in the ability of these species to completely invade a new habitat and the ability to form a stable population (Nešić and Bjedov,

2021). Smaller seeds can adjust quicker in disturbed habitats and disperse over long distances (Nešić et al., 2022). In addition, smaller seeds can remain viable for longer in the soil, form permanent soil seed banks, and more often escape predation (Thompson et al., 1993; Moravcova et al., 2010; Masole et al., 2022; Huang et al., 2023) while large seeds have a greater advantage in seed germination and seedling growth stages (Huang et al., 2023).

The success of invasive species outside of their native range may be attributed to an escape from natural enemies, which also applies to seeds (Keane and Crawley, 2002). Most seed-producing plants have insects (seed predators) associated with their seeds which place some level of control on them (Keane and Crawley, 2002). However, when there is a scarcity of natural enemies, and environmental conditions are suitable, there is a rapid increase in the abundance and distribution of seeds, resulting in plants becoming invasive (Keane and Crawley, 2002). Some species, once in their invaded range, may also allocate more resources to seed production compared to when growing in their native range, promoting faster spread (Kwong et al., 2019).

There are classical weed biological control programmes where biocontrol agents are deliberately introduced to IAPs to control their populations via seed predation (Impson et al., 2021; Zachariades, 2021). Seastedt's (2015) findings showed that herbivores and pathogens can control invasive plant species although this program still needs to be discussed and expanded. For example, although rodents are known to predate on seeds, they are also known to regulate the establishment of native and invasive plants (Pearson et al., 2018; Taylor et al., 2020). However, seed predation by seed wasps has been shown to negatively impact seed production of *Euphorbia* species, with higher losses reported in periods of low seed production (Boieiro et al., 2020).

2.1.3. Reproductive ecology of invasive Rosaceae in South Africa

Several invasive Rosaceae species are becoming dominant and problematic in the Afromontane grasslands of South Africa (SA). To determine what may be facilitating these invasions, Moloi (2022) and Adams et al. (2023) investigated the importance of seed ecology in the invasion and spread of two Rosaceae species, *Pyracantha angustifolia* (Franch.) C. K. Schneid. and *Cotoneaster pannosus* Franch. Both are pollinated by generalist insects and produce high numbers of seeds in red or orange-coloured fleshy fruits dispersed by local frugivores such as birds and mammals (Moloi, 2022; Adams et al., 2023). Floral visitor exclusion experiments demonstrated that *P. angustifolia* could set seeds without pollen vectors (Adams et al., 2023). Assessment of fruit and seed production for both species revealed an exponential relationship between seed set and plant size with high natural seed yield (Moloi, 2022; Adams et al., 2023). Soil core samples showed high seed densities underneath the shrub canopies, with significantly decreasing densities as the distance from the shrub increased (Moloi, 2022; Adams et al., 2023). Seeds of *P. angustifolia* buried in the soil survived for less than six months, which suggests this species has low survival rates for re-establishment through the soil seed bank (Adams et al., 2023). In contrast, Moloi (2022) showed that the seeds of *C. pannosus* were able to survive longer than two years in the soil. These studies also showed that Rosaceae species produce high numbers of seeds that are correlated to the size of the shrub, and seeds are found in higher numbers underneath the shrub and decrease in number as distance from the shrubs increases. However, neither of these studies considered that they might be facilitating each other, and other seedlings, despite being seen in dense mixed stands.

A third common species invading these grasslands is *Rosa rubiginosa* L. Currently, *R. rubiginosa*'s reproductive ecology, including the number of seeds produced and their viability in this montane system, remains unstudied.

2.1.4. Reproduction of *Rosa rubiginosa*

In general, *R. rubiginosa* is known to have delayed germination and it can take up to two years for the seeds to germinate (Huxley, 1992). This delay may be due to the hard pericarp of their seeds, the inhibitors in the pericarp, and physiological barriers in the embryo (Zhou et al., 2009). This is not uncommon in the Rose family as rosaceous species are known to exhibit difficulties in seed germination due to strong dormancy (Younis et al., 2007). In a commercial setting, this deep seed dormancy makes it challenging to establish commercial plantations from seed (Haouala et al., 2013; Mokhobo, 2018). However, seed dormancy plays a critical role in ensuring the successful development of seeds and readiness for germination, since it postpones seed germination until all conditions are favourable (Graeber et al., 2012). Long periods of dormancy can be advantageous for a species becoming invasive (Hofmann, 2014), especially in disturbance-prone landscapes where there are natural disturbances (e.g., fire or herbivory) (Cully et al., 2003).

Deep dormancy in *R. rubiginosa* is also associated with higher concentrations of an inhibiting hormone called abscisic acid in the pericarp and testa (Hartmann et al., 2002). There are different treatments that have been investigated to try and break the seed dormancy of *Rosa canina* L. and other *Rosa* species (Mokhobo, 2018). For example, Mokhobo (2018) trialled several germination techniques, including applying hot water and sulphuric acid treatments, and warm and cold stratification, all of which were unsuccessful in breaking seed dormancy. In addition, the study did not investigate the seed viability of *R. rubiginosa* in the field. According to Westwood (2020), *R. rubiginosa* shrubs mostly invade areas that experience over 100 annual frost days suggesting that cold temperatures are needed for the success of the dispersal of this shrub, a factor that can be used to predict where it could potentially spread.

Currently, despite being one of the dominant invasive species in the colder regions of SA, there are no ecological studies that have been conducted on *R. rubiginosa* in SA. Despite some data showing seed germination is affected by cold stratification (Milberg and Andersson, 1998; Olmez, 2007), it hasn't been determined whether this is contributing to the plant's invasion success in SA. Furthermore, whether there are sufficient cold days in invaded regions of SA to break seed dormancy is undetermined. Following other Rosaceae species (e.g. Adams et al., 2021; Moloi et al., 2022), it is suspected that *R. rubiginosa* produces a high number of seeds, which may be driving its invasion into the grasslands. However, the exact number of seeds entering the environment, where those seeds end up, what becomes of them once they enter the soil bank, and how viable they are, have not been investigated. This information could assist land managers with developing effective management practices for the plant.

In SA the shrub is predated upon by a non-native seed-feeding wasp, *Megastigmus aculeatus* Swederus (Hymenoptera: Torymidae) (Swederus, 1795), that was introduced with its host, *R. rubiginosa*, from Eurasia (Doğanlar, 2015). Family Megastigmidae Thomson, (1876) is a family within the superfamily Chalcidoidea, which only has phytophagous species (Van Noort, 2024). *Megastigmus aculeatus* is a specific predator of the seeds of *Rosa* species and it is known to be a pest of the commercial Rose industry in Europe (Auger-Rozenberg and Roques, 2012; Doğanlar, 2015; Zerova et al., 2021). It is expected that the wasp species might be limiting or at least reducing the spread of *Rosa* species through seed predation in SA.

2.1.5. Aims and objectives

This chapter aimed to investigate the seed biology of *R. rubiginosa* in the eastern great escarpment in the eastern Free State province of SA by determining the germination period, the necessity of cold stratification to break seed dormancy, soil seed bank, seed viability/longevity, seed and fruit production, and seed predation rates by *M. aculeatus*. It is

hoped that results from this study may identify potential stages of the life cycle of *R. rubiginosa* which could be targeted by management options. This was achieved through the following objectives: 1) determined the germination rate and importance of cold stratification for seed germination of *R. rubiginosa* seeds; 2) determined the extent of *R. rubiginosa* seed survival in the soil; 3) determined the extent of the soil seed bank through soil cores; 4) determined the fruit and seed production of *R. rubiginosa*; and 5) determined the impact of the seed-predating wasp *M. aculeatus* on *R. rubiginosa* in the eastern Free State.

2.2. Materials and methods

2.2.1. Study sites

Four separate sites were selected (Figure 2.1 and Figure 2.2) for the study, which are privately owned farms in Clarens, eastern Free State province, SA. All the sites are open grasslands occasionally grazed by livestock. The first site has a tributary of the small Caledon River flowing through it (S 28°32.844, E 28°25.215; Clifton farm site 1; Figure 2.1 site 1 and Figure 2.2 A). The second site is dominated by grasses and native *Leucosidea sericea* Eckl. & Zeyh. (S 28°54.067, E 28°42.927; Clifton farm site 2; Figure 2.1 site 2 and Figure 2.2 B). The third site is on a rocky hillside (S 28°29.003, E 28°49.443; Rockhill farm; Figure 2.1 site 3 and Figure 2.2 C). The fourth site is also dominated by grasses and is near a swamp (S 28°32.233, E 28°30.463; De-Rots farm; Figure 2.1 site 4 and Figure 2.2 D). The studied sites are in the same region as Chapter 3, please see Chapter 3 for site characteristics such as vegetation structure, temperature, etc. At Clifton farm site 1 there were ~ 1.8 *R. rubiginosa* per 10 m^2; Clifton farm site 2 had ~ 5 *R. rubiginosa* per 10 m^2, and Rockhill farm had ~ 1.6 *R. rubiginosa* per 10 m^2 (Figure 2.3).

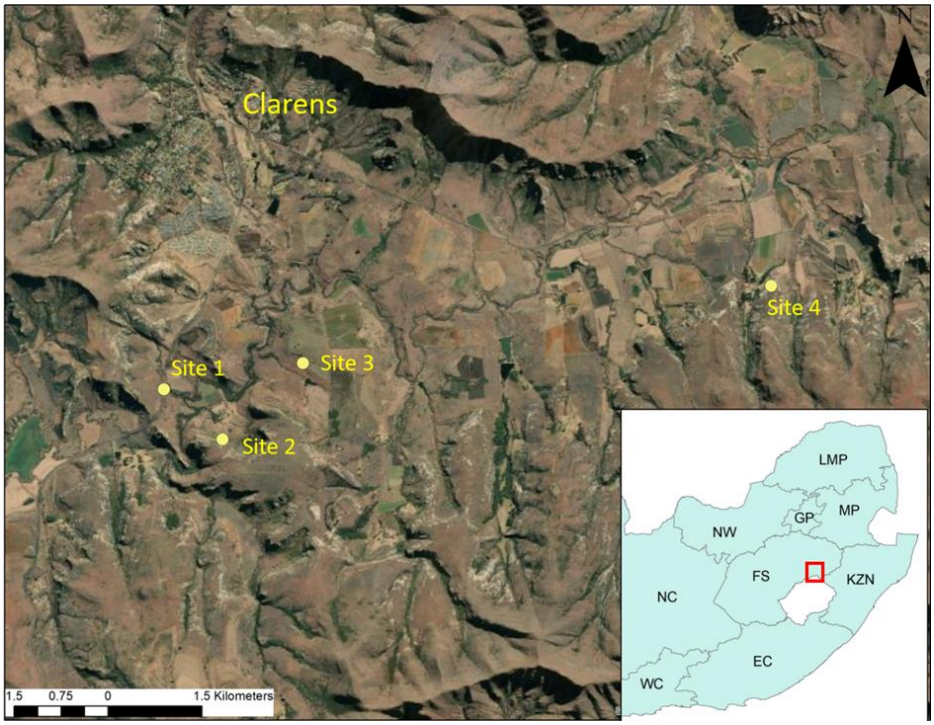

Figure 2.1: Map showing the location of the four study sites (Site 1: Clifton farm site 1 (S 28°32.844, E 28°25.215), Site 2: Clifton farm site 2 (S 28°54.067, E 28°42.927), Site 3: Rockhill farm (S 28°29.003, E 28°49.443), and Site 4: De-Rots farm (S 28°32.233, E 28°30.463)) all situated near the town of Clarens, eastern Free State province, South Africa.

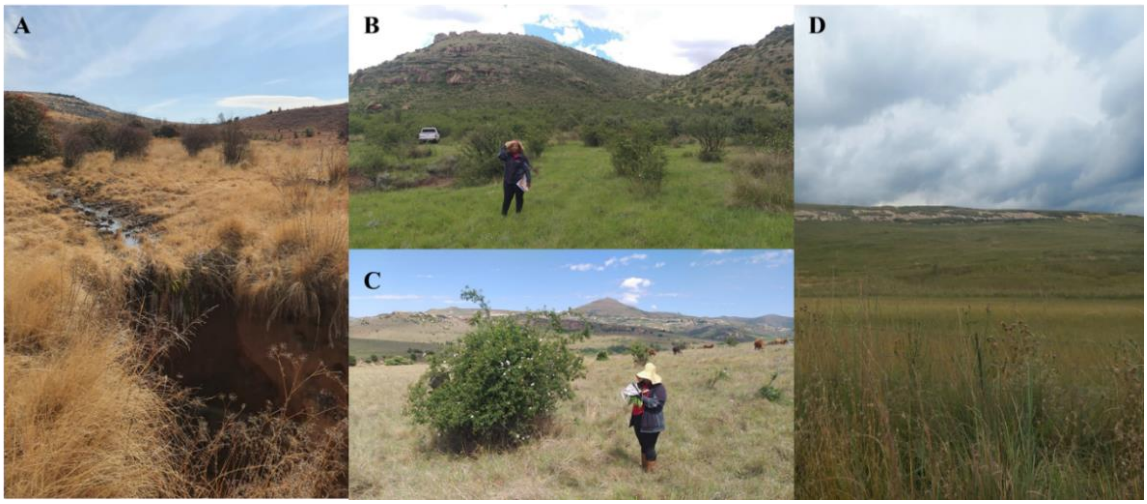

Figure 2.2: Photos showing the vegetation structure and *Rosa rubiginosa* shrubs at the study sites: (A) Clifton farm site 1; (B) Clifton farm site 2; (C) Rockhill farm; (D) De-Rots farm, all

around the town of Clarens in the eastern Free State province, South Africa. Photo credit B & C: G.D Martin.

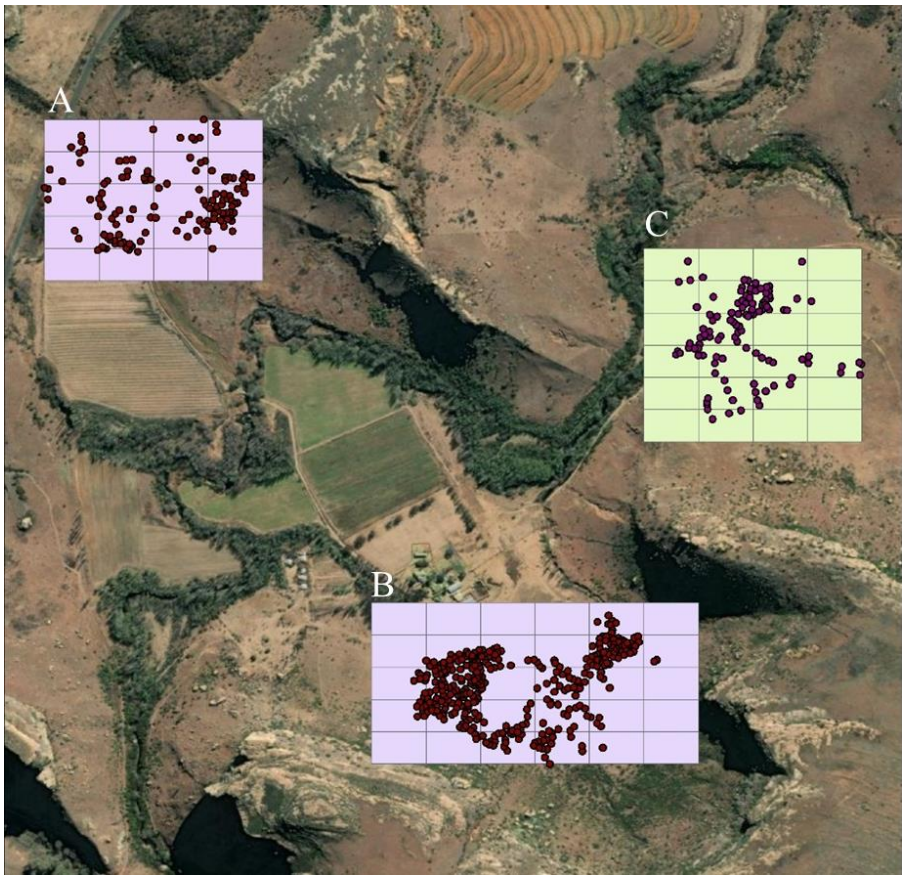

Figure 2.3: Occurrence records of *Rosa rubiginosa* shrubs (red dots) at three of the study sites, namely (A) Clifton farm sites 1 and (B) 2, and (C) Rockhill farm. Coloured squares represent a 100 x 70 m fishnet created in Arcmap10.3.

2.2.2. Germination trials

The aim of this experiment was to determine the importance of cold stratification for *R. rubiginosa* seed germination, as it is suggested that the species is climatically limited to the colder regions of SA (Martin, 2021). To assess the effect of cold stratification, the germination rates of seeds from the following treatments were measured: naturally collected fruits exposed to the cold eastern Free State winter; seeds stored in a refrigerator (6 °C); and seeds stored at ambient temperature (control). In April 2021 approximately 100 mature rosehips (fruit of a

rose) from randomly selected *R. rubiginosa* shrubs were collected from all four study sites. Seeds were manually removed from the fruit pulp and skin, and 800 seeds from harvested fruits were randomly selected. Seeds were soaked in distilled water for 24 hours and dried using a paper towel. The seeds were then divided into two groups: the first group of 400 seeds was placed in a polyethylene bag in the refrigerator (6 °C); the second group of 400 seeds was placed in a polyethylene bag and left at ambient temperature (control). Every two weeks thereafter, for a total of 12 weeks (May 2021 to October 2021), 20 seeds were removed from the refrigerator (effectively giving seeds an additional 14 days of cold stratification between experimental trials), 20 seeds from the control treatment (stored in a laboratory at ambient temperature), and 20 seeds were collected directly from shrubs in the field (soaked in distilled water for 24 hours before proceeding with experiment; exposed to natural cold temperatures in the wild). All these three treatments were placed in three separate petri dishes and stored at indoor ambient temperature. Petri dishes were prepared by placing a double layer of filter paper in the dish. Collected seeds were placed between the filter paper in the petri dishes and moistened with distilled water until the whole filter paper was moistened. All petri dishes were placed in a dark cabinet at ambient temperatures. Petri dishes were checked every two to three days for germinated seeds and sprayed with water (moistened to keep the filter paper from drying), if necessary, to prevent the filter paper and seeds from drying out. Filter papers were changed as necessary when stained by oil from *R. rubiginosa* seeds. Monitoring was done for two years (May 2021 - May 2023). A total of twelve sets of fruit, and therefore seed samples, were collected over the 2021 fruiting season until the flowering period of *R. rubiginosa* started. The number of seeds that germinated from each collection time and treatment was used to calculate mean ± SE (standard error) and graphed using Microsoft Excel.

2.2.3. Seed viability/longevity

This experiment aimed to determine the viability and longevity of *R. rubiginosa* seeds in the soil seed bank. This was done by burying seeds on two sites a north-facing (De-Rots farm) and South south-facing site (Clifton farm site 1). Rosehips fruits (approximately 400) were harvested from November 2021, from approximately 50 *R. rubiginosa* shrubs from Clifton farm site 1. The flesh and skin were removed by hand, and the seeds were washed with distilled water and dried with a paper towel to remove hairs found inside the fruit. Leslie (1954) recommends that seeds of Rosaceae species be stored in dry-sealed containers in a cool place. Approximately 4 000 seeds from the rosehip fruits collected at Clifton farm site 1 were cleaned and stored in a cool, dry, dark container in a cupboard for two months. Following storage, 100 randomly selected seeds were tested for viability. The remaining seeds were randomly divided into 36 batches of 100 seeds and each batch was placed into one of 36 premade envelopes made from 60% nursery shade cloth (10 x 5 cm; Figure 2.4 A). Due to deep dormancy in these seeds, the International Seed Testing Association (ISTA) recommends the use of tetrazolium staining rather than germination tests for the evaluation of seed quality (ISTA, 1993). Seeds were first soaked in distilled water for 24 hours, followed by removing the distal third of the seeds with a transverse cut, and then soaked in a 1% solution of tetrazolium solution for 48 hours. Viable seeds-stained pink completely, but seeds were considered to still be viable if only the radicle tip and the distal third of the cotyledons were stained (ISTA, 1993). At each site, seed-containing envelopes were buried, in one of three holes (replicates), with each replicate comprising six envelopes. Envelopes were buried at a depth of 10 cm and the burial sites were marked with red metal stakes (Figure 2.4 B). One envelope was removed from each replicate after two, four, eight, and eighteen months. Seeds from each envelope were then tested for viability with tetrazolium staining. A one-way ANOVA with a Tukey HSD *post-hoc* test was conducted to compare the differences in the number of viable seeds between sites and the time

periods, and a regression test was conducted to assess the relationship between seed viability (%) and the length of burial. Data were analaysed in IBM SPSS Statistics version 29 and graphed using Microsoft Excel (data were normally distributed, Shapiro-Wilks Normality Test, $P > 0.05$).

Figure 2.4: A) Black nursery shade cloth envelopes containing 100 *Rosa rubiginosa* seeds each, and the typical hole where seeds were buried at the two sites at Clifton farm site 1 and De-Rots farm. B) Red metal stakes (black arrow) were used to mark holes in the veld so they could be easily located.

2.2.4. Soil seed bank

To determine the density of seeds in the soil, soil core samples were collected from underneath twenty *R. rubiginosa* shrubs at each of three sites (Clifton farm sites 1 and 2, and Rockhill farm). Soil core samples were collected using a soil auger (7.5 cm diameter x 20 cm depth: Figure 2.5 B). At each shrub, three cores were taken in two directions at an increasing distance from the shrub: under the canopy, 2 m, and 8 m away from the base of the shrub. Soil from the soil auger was sieved through a 1 mm sieve (Figure 2.5 A). The debris left in the sieve was

collected and placed in labelled paper bags to be sorted. In the laboratory, the debris was carefully searched for *R. rubiginosa* seeds which were collected and counted. *Rosa rubiginosa* produces numerous seeds which appear yellow, are 4.5 - 6 mm long, and irregularly angled (Parsons and Cuthbertson, 2001), but when seeds are stored in the soil, they change colour to a brownish-black colour. Seed counts in the soil were compared between the different distances from the shrub canopy with a Kruskal Wallis H-Test,with pairwise comparisons, in IBM SPSS Statistics version 29, and graphed using Microsoft Excel (Data were not normally distributed, Shapiro-Wilks Normality test, $P < 0.05$).

Figure 2.5: Photographs showing the processes of A) sieving soil collected using a soil auger, and B) digging up a soil sample using an auger. Photo credit: (A) N Radebe; (B) K.T Moloi

2.2.5. Fruit and seed production

The number of fruits and seeds produced by *R. rubiginosa* shrubs was determined in relation to shrub size. This was done on three sites (Clifton farm sites 1 and 2, and Rockhill farm). Shrub size was determined by measuring the height of the shrub from ground level to its highest

point (in m), and two widths were measured at the widest part of each shrub in North to South and East to West directions. The compass points divided the shrub into four equal quadrants (SE, NE, SW, NW), to assist with accurately counting the number of fruits. The number of fruits in each quadrant was counted and added together as the total number of fruits per shrub. To analyse the data of this experiment, data were square-root transformed as untransformed data were not normally distributed (Shapiro-Wilks Normality test, $P < 0.05$).

The number of seeds produced by a *R. rubiginosa* shrub was then determined by counting the number of seeds per fruit from 200 fruits randomly collected from different shrubs and then calculated the average number of seeds per fruit. The average number of seeds per fruit was multiplied by the number of fruits produced per shrub to estimate the total number of seeds produced by a shrub. Shrub volume was calculated as a cylinder, using the formula volume (m^3) = $\pi r^2 h$. A linear regression analysis of the relationship between shrub size and fruit production or total seed production per shrub for each site and all three sites combined was performed in Microsoft Excel.

2.2.6. Seed predation of *Rosa rubiginosa*

This experiment was done to determine the impact (percentage of seed destroyed) of the seed predator *M. aculeatus* on *R. rubiginosa* seeds. One hundred rosehip fruits were randomly collected every month from March 2021 - September 2021 from different shrubs until fresh fruits were no longer available from Clifton farm site 1. Once collected from the field, 20 rosehips were then placed in each of five emergence jars (100 ml) and each jar was covered with gauze and tightened with a rubber band. The jars were then stored in an insect tent (bug dorm) in the laboratory at room temperature. The laboratory has no curtains, so it experienced natural day:night conditions. Jars were checked every two to three days and any wasp emergence was noted. All emerging *M. aculeatus* were counted. The percentage of seeds predated, was calculated as the number of seeds per jar divided by the number of *M. aculeatus*

wasps that emerged, multiplied by 100. This was calculated per jar according to their collection date, then totalled to calculate the final average percentage of seeds predated. The number of wasps that emerged from collected seeds was compared among collection months with a Kruskal Wallis H-Test and pairwise comparisons in IBM SPSS Statistics version 29 and graphed using Microsoft Excel (data were not normally distributed, Shapiro-Wilks Normality test, $P < 0.05$). In addition, four paper bags of approximately 100 000 *R. rubiginosa* seeds were received from a local Rosehip oil producer who had received seeds harvested in 2021 from SA and Lesotho. *Megastigmus aculeatus* wasps had already emerged from the seeds. These seeds were collected from ~ 50 sites spanning the two countries and amalgamated at the factory (Rosehip oil producer, pers. comm.). To determine the number of damaged seeds in the seeds received, where wasps had already emerged, 100 seeds were randomly selected five times (500 seeds in each bag (2000 seeds)) and checked for the presence of emergence holes (Figure 2.6).

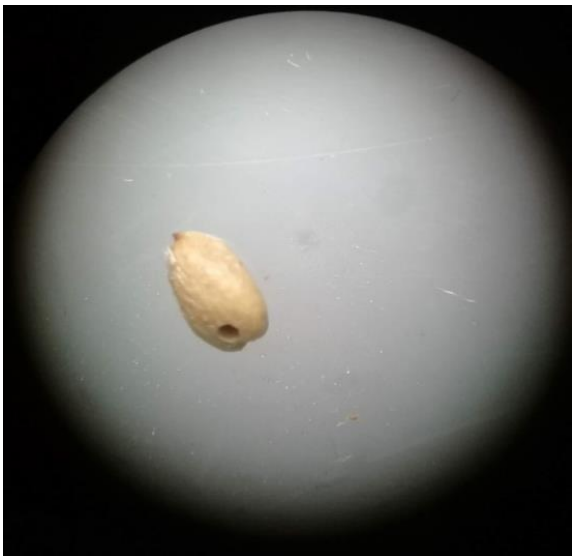

Figure 2.6: A *Rosa rubiginosa* seed from which a *Megastigmus aculeatus* wasp had emerged, shown by the small emergence hole viewed under a microscope (length of seed is 4 mm).

2.3. Results

2.3.1. Germination trials

Germination trials were conducted to assess the germination rate and importance of cold stratification for seed germination of *R. rubiginosa*. A total of 720 seeds were tested for germination from the original sample of 800 seeds. Findings showed that out of all the treatments used (fridge temperature, room temperature, and field collected), only seeds that were exposed to cold stratification via a refrigerator germinated (Figure 2.7). The number of germinated seeds from fridge temperature was extremely low, with only nine out of 720 seeds (1.25%) germinated (Figure 2.8). The first germinated seed since the experiment started, was observed after nine months while the last observed seed germinated after 24 months, after which the study was terminated. Seeds that germinated from the refrigerator took an average time of 16.7 ± 1.9 months (mean ± SE) to germinate. No seeds that were stored at room temperature or collected from the field germinated over the 24-month period.

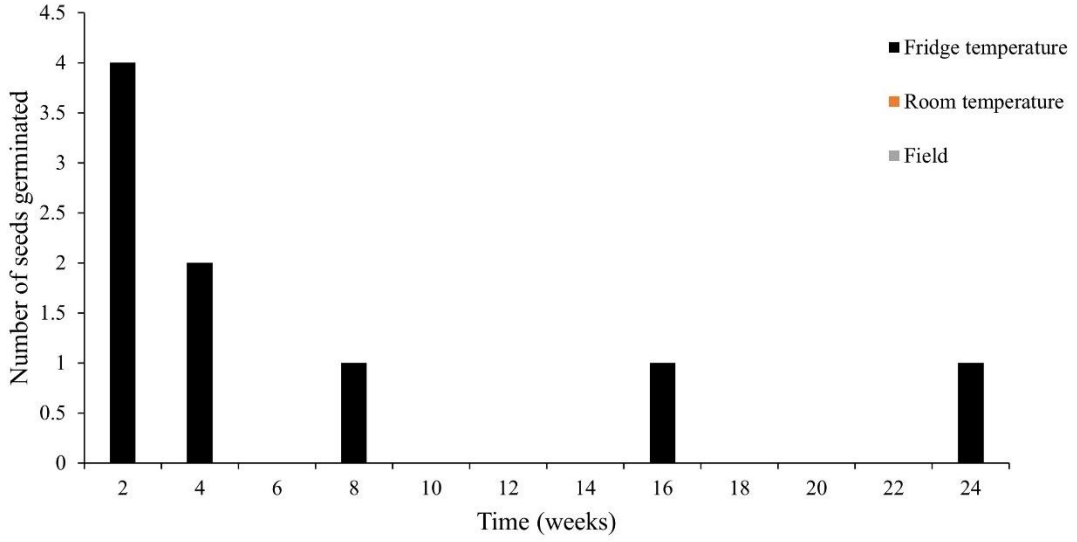

Figure 2.7: Number of *Rosa rubiginosa* seeds from each collection period that germinated from the seeds that were stored in fridge temperature, room temperature, and field collected.

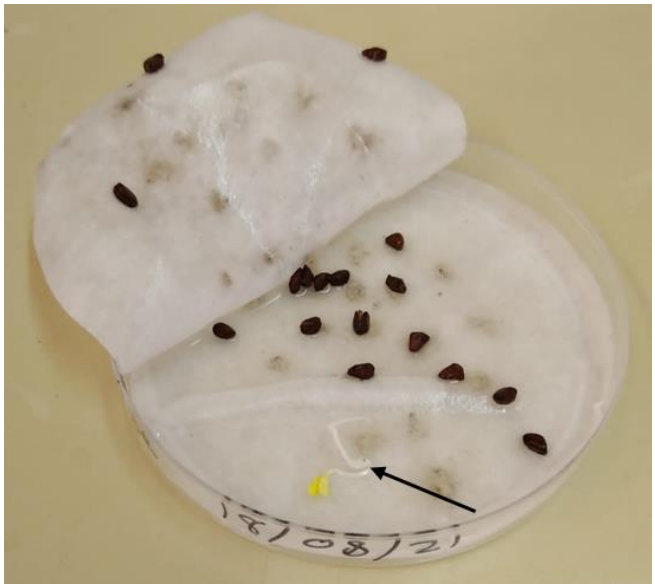

Figure 2.8: Petri dish with 19 ungerminated seeds and one germinated *Rosa rubiginosa* seed from the refrigerator treatment.

2.3.2. Seed viability/longevity

This experiment was conducted to investigate the seed viability of *R. rubiginosa* seeds after being buried for different periods. The viability of the seed stock prior to burial was 90%. Overall, there were significant differences in the percentage of viable seeds among the different time periods for which seeds were buried in the soil ($F_{3,20} = 22.577$; $P < 0.05$; Figure 2.9).

Results for the combined sites showed that there were no significant differences in viability between seeds buried for two and four months with almost a constant seed viability percentage of 88% and 89% respectively. After eight months seed viability percentage increased (96%) but decreased after 17 months (83%), this month had the lowest seed viability percentage out of all months presented and was significantly different from every other month.

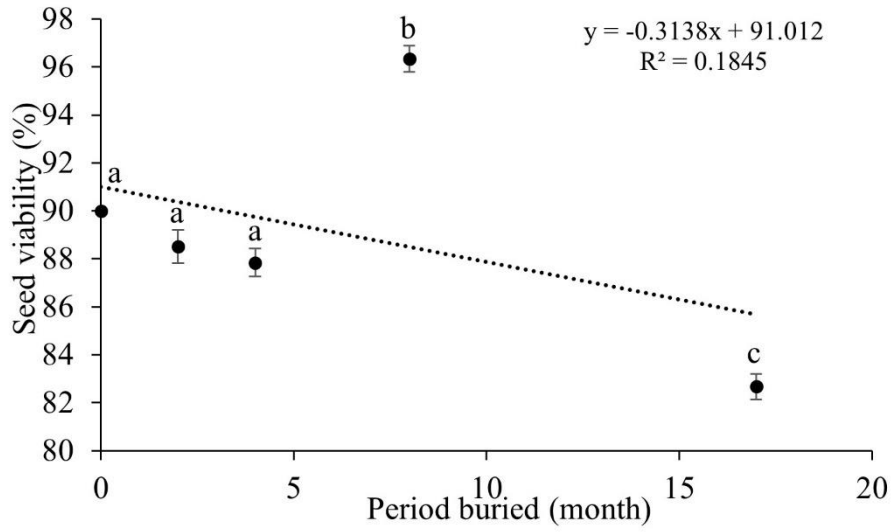

Figure 2.9: Mean ± standard error of seed viability percentages of *Rosa rubiginosa* seeds at De-Rots farm and Clifton farm site 1 (combined) after burial for different amounts of time (months). Different letters above the bars indicate significant differences.

2.3.3. Soil seed bank

To determine the number of *R. rubiginosa* seeds stored in the soil, the number of seeds in soil core samples was taken at increasing distances from parent shrubs. The soil cores contained intact, decayed, and damaged seeds, and occasionally whole fruits. However, only intact seeds were counted. Significantly more seeds were found directly under the parent plant compared to 2 and 8 m from the parent shrub (Pairwise comparison, $P < 0.05$). An average of 145 ± 65 (Mean ± SE) *R. rubiginosa* seeds per m^3 were collected directly under the parent shrub, while 123 ± 5 (Mean ± SE) seeds per m^3 were collected at 2 m away, and at 8 m away 5 ± 7 (Mean ± SE) seeds per m^3 were collected ($H = 17.91$; $df = 2$; $P < 0.05$; Figure 2.10).

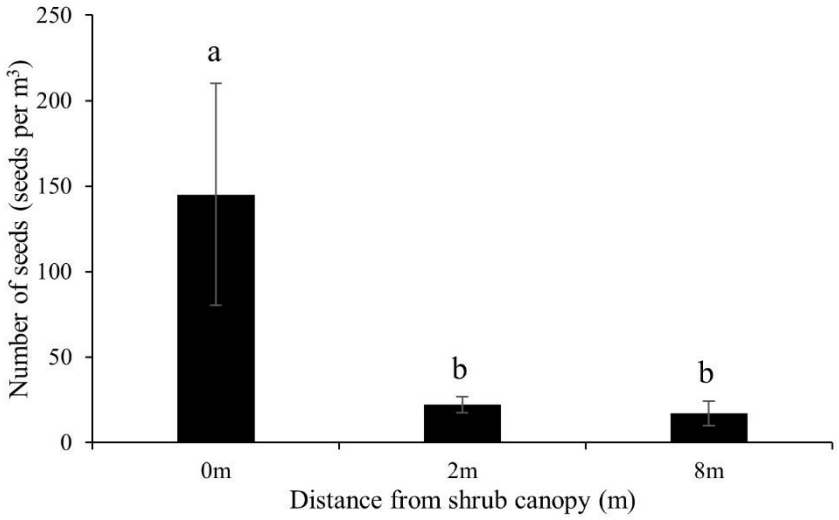

Figure 2.10: Number of *Rosa rubiginosa* seeds per m^3 (mean ± standard error) in the soil seed bank at different distances from the shrub. Different letters above the bars indicate significant differences ($P < 0.05$).

2.3.4. Fruit and seed production

This experiment was done in March 2023, while the rosehips were still fresh on the shrub, to determine the rosehip production of *R. rubiginosa* in relation to shrub size. Clifton farm site 1 showed a positive relationship between shrub size and fruit production ($R^2 = 0.657$; $F_{1.18} = 34.431$; $P < 0.05$; Figure 2.11). This site had an average shrub size of 23 ± 4 m^3 and an average of 298 ± 61 fruits per shrub. The highest number of fruits produced by a shrub was 916 fruits from a shrub with a volume of 45 m^3. The shrub with the highest volume of 77 m^3 produced 751 fruits. The smallest number of fruits and shrub volume measured was from a shrub with a volume of 1 m^3 which produced 17 fruits.

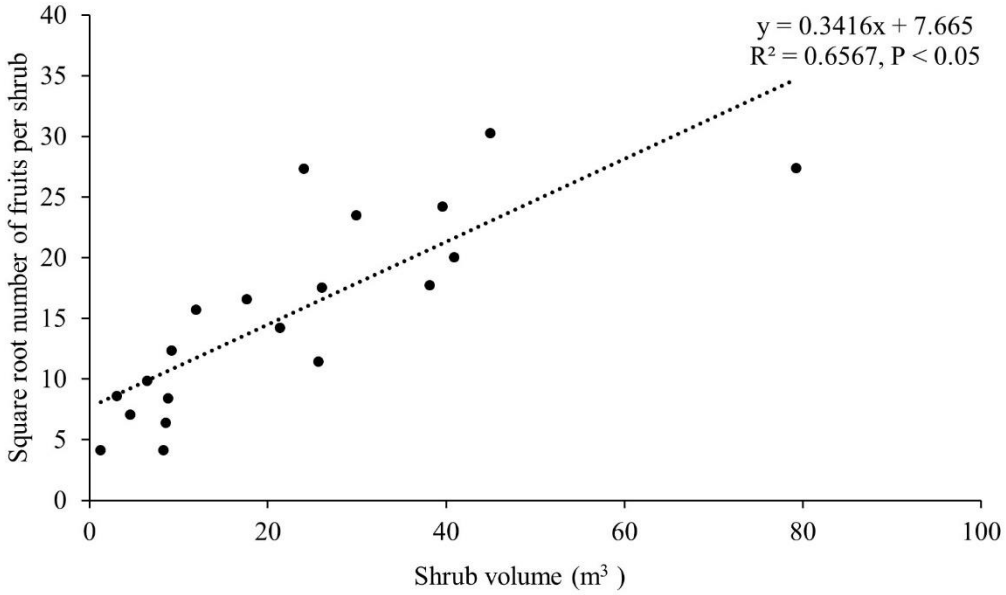

Figure 2.11: A linear regression showing the relationship between shrub volume (m^3) and fruit production (square root of number of fruits per shrub) of *Rosa rubiginosa* shrubs (n = 20) for the 2023 fruiting season at Clifton farm site 1 in Clarens.

There was a positive relationship between shrub size and fruit production at Clifton farm site 2 ($R^2 = 0.8$; $F_{1,18} = 72.161$; $P < 0.05$; Figure 2.12). This site had an average shrub size of 15 ± 3 m^3 and an average of 288 ± 62 fruits per shrub. The highest number of fruits produced by a shrub was 898 fruits with a shrub volume of 52 m^3; this was also the largest shrub at the site. The smallest shrub had a volume of 1 m^3 and produced four fruits.

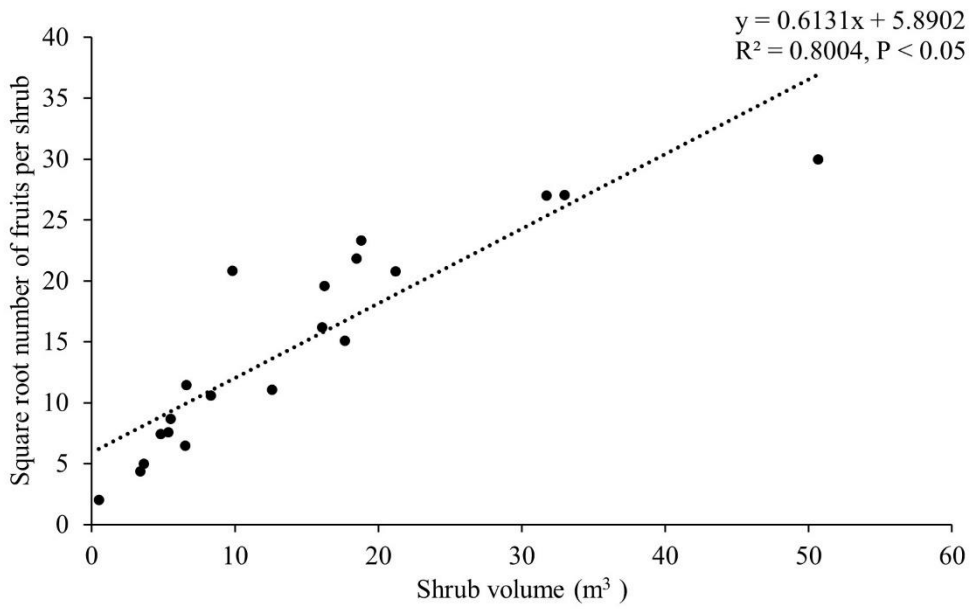

Figure 2.12: A linear regression showing the relationship between shrub volume (m^3) and fruit production (square root of number of fruits per shrub) of *Rosa rubiginosa* shrubs (n = 20) for the 2023 fruiting season at Clifton farm site 2 in Clarens.

There was a positive relationship between shrub size and fruit production at the Rockhill farm ($R^2 = 0.497$; $F_{1,18} = 17.764$; $P < 0.05$; Figure 2.13). This site had an average shrub size of 39 ± 7 m^3 and an average of 853 ± 110 fruits per shrub. The highest number of fruits produced by a shrub was 1 950 fruits with a shrub volume of 78 m^3. The biggest shrub had a volume of 122 m^3 and produced 1 130 fruits. The smallest number of fruits produced by a shrub was 118 fruits, for the smallest shrub volume was recorded of 5 m^3.

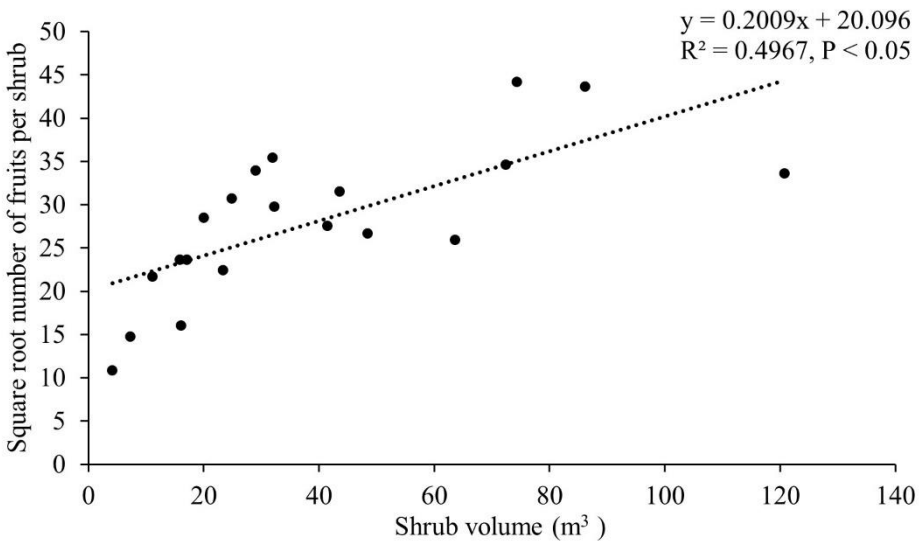

Figure 2.13: A linear regression showing the relationship between shrub volume (m^3) and fruit production (square root of number of fruits per shrub) of *Rosa rubiginosa* shrubs (n = 20) for the 2023 fruiting season at Rockhill farm in Clarens.

Analysis of both fruit and seed production from the combined data of three different study sites, namely Clifton farm sites 1 and 2, and Rockhill farm showed a positive relationship between shrub volume and fruit production ($R^2 = 0.601$; $F_{1,58} = 88.426$; $P < 0.05$; Figure 2.14). These combined sites had an average shrub size of 25 ± 3 m^3 and produced an average of 480 ± 57 fruits per shrub. Out of all *R. rubiginosa* shrubs studied at three sites, Rockhill farm was the farm that had the largest shrub volume and produced the highest number of fruits.

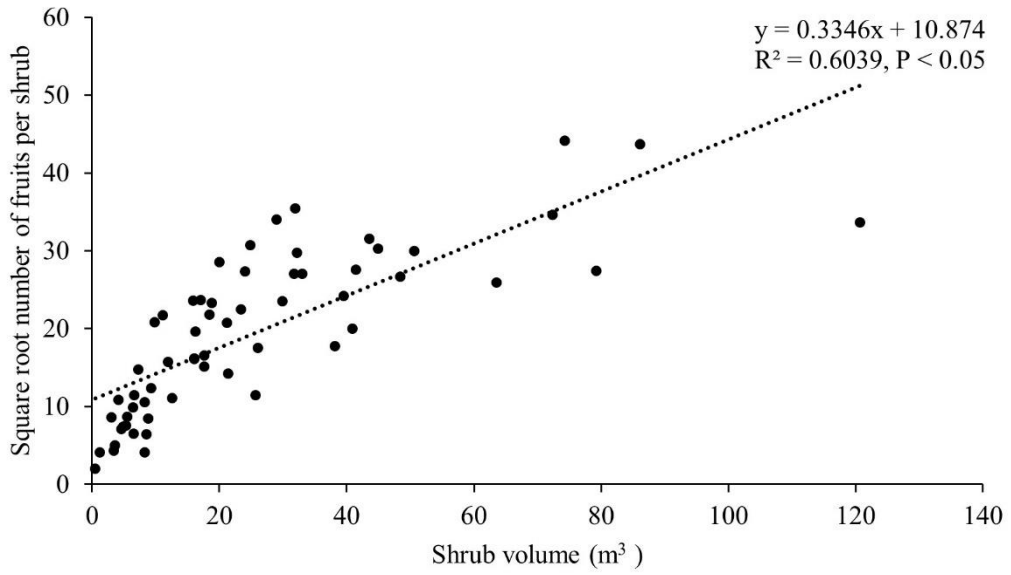

Figure 2.14: Linear regression showing the relationship between shrub volume (m^3) and the square root of number of fruits produced by *Rosa rubiginosa* shrubs (n = 60) for the 2023 fruiting season for three combined sites (Clifton farm sites 1 and 2, and Rockhill farm) in Clarens.

We counted total number of seeds per fruits to range between a minimum of one seed per fruit to a maximum of 38 seeds per fruit. Average number of seeds per fruit was calculated as 18 seeds. We used the calculated average number of seeds per fruit to estimate number of seeds produced per shrub, by multiplying it by the number of fruits produced per shrub. The counted minimum number of seeds produced was 72 seeds per shrub with a volume of 1 m^3, which also represented the smallest shrub volume. The shrub with the largest volume of 122 m^3 produced 2 196 seeds. The shrub that produced the most seeds produced 22 608 seeds and had a volume of 41 m^3. When analysing the relationship between shrub volume and the total number of seeds per shrub, as similar result was achieved for fruit produced per shrub, (Figure 2.15), this was due to using the same average number calculated to convert to seeds produced per shrub.

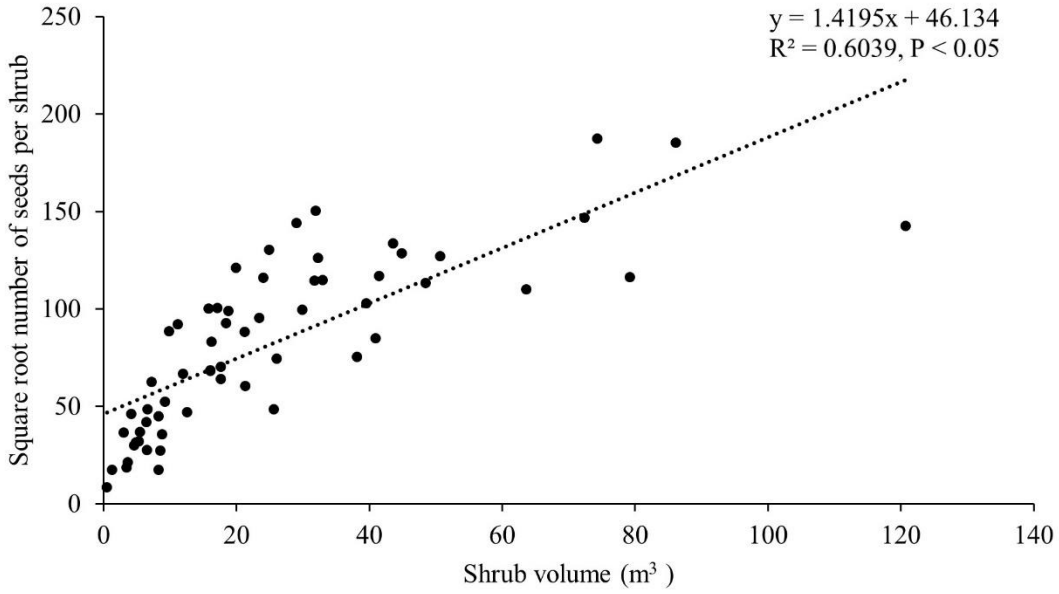

Figure 2.15: Linear regression showing the relationship between shrub volume (m^3) and the square root of the total number of seeds produced by *Rosa rubiginosa* shrubs for the 2023 fruiting season for all three sites combined in Clarens.

2.3.5. Seed predation of *Rosa rubiginosa*

Megastigmus aculeatus is the only known seed parasitoid of *R. rubiginosa* seeds in SA, and this study aimed to determine the impact of this seed predator (percentage of seed destroyed). A total of 148 *M. aculeatus* wasps emerged from the 700 fruits collected between March and September 2021, giving an overall percentage of 1.28% of seeds predated. The first emergence of wasps was observed on 28 September 2021, and the last wasp was observed on 29 October 2021. Significantly more wasps emerged in seeds collected in June and July 2021 ($H = 16.52$; $df = 6$; $P < 0.05$; Figure 2.16). The highest number of *M. aculeatus* wasps was collected in July 2021 (74 *M. aculeatus* wasps) followed by June 2021 (45 wasps). The number of wasps that emerged ranged from one wasp to 44 wasps in different emergence jars collected at different times. No wasps emerged from samples collected in April, May, and August 2021.

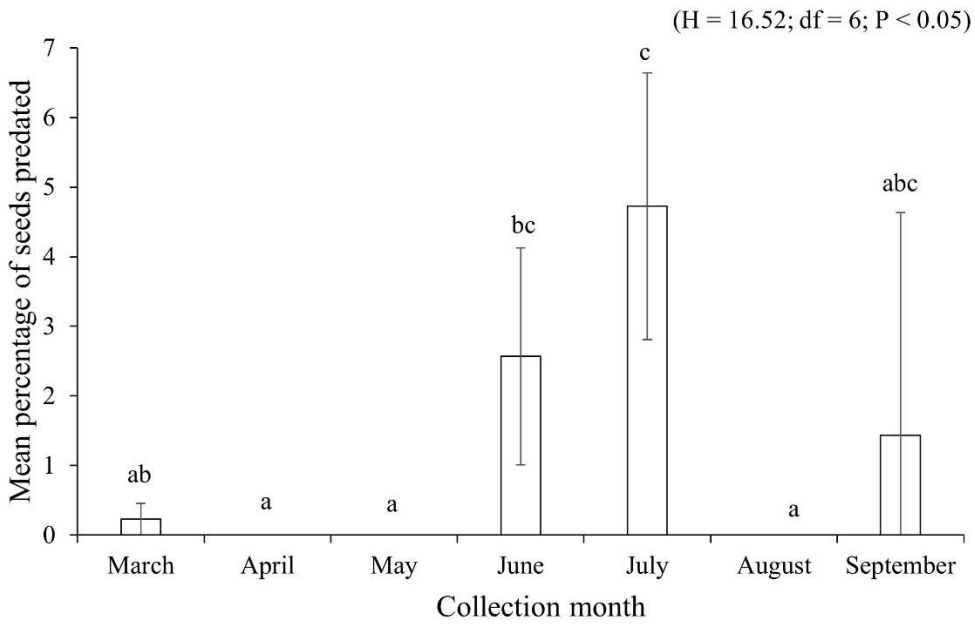

Figure 2.16: Percentage of *Rosa rubiginosa* seeds (mean ± standard error) predated by *Megastigmus aculeatus* in 2021, from fruits collected during different months of the year ($P < 0.05$).

Seeds collected from a local Rosehip oil producer revealed that out of 2 000 seeds sampled (four samples of 500 seeds), only 78 seeds had *M. aculeatus* emergence holes, suggesting that only 3.9% of *R. rubiginosa* seeds are impacted by seed predation.

2.4. Discussion

This study has shown that in the grassland biome of SA, the invasive alien *R. rubiginosa* produces a high number of seeds (22 608 seeds) per shrub and produces, on average, 18 seeds per fruit (range = 1 to 38 seeds per fruit); this is more than other alien Rosaceae from a similar region. Rosaceae species such as *C. pannosus* produce two seeds per fruit (Moloi, 2022) and *P. angustifolia* produces a range of 3 - 6 seeds per fruit (Adams et al., 2023). These seeds fall to the ground where they become part of the seed soil bank. This study has shown that *R. rubiginosa* seeds are able to survive for a long period of time under the soil (> 18 months). Germination is an important factor for any plant to spread. However, the hard pericarp of the

R. rubiginosa seeds, while protecting them effectively in the seed bank for long periods, also makes determining the exact mechanisms that may trigger germination difficult. While results suggest cold temperature may be important in triggering germination, further studies are required. Further studies may include studying to what extent root suckers facilitate the spread of *R. rubiginosa* shrub since the current study established seeds take time before germinating. Studies to check whether seeds of *R. rubiginosa* germinate faster when they are in soil than when treated in the laboratories are also recommended.

2.4.1. Germination trials

Germination is a fundamental step in a plant's life cycle, and invasion success has been ascribed, amongst other factors, to high germination rates (Radford and Cousens, 2000; Mandák, 2003). Temperature variation, water, and genetics are some factors that can break the intensity of seed dormancy (Baskin and Baskin, 2014; Radoglou, 2015). It is well-documented that the *Rosa* genus is associated with low germination (Anderson and Byrne, 2005; Younis et al., 2007). Within the genus, *R. rubiginosa* seeds are known to have deep dormancy (Hu et al., 2018; Iwasaki et al., 2022). Several methods have been used to break rose seed dormancy, including a combination of warm and cold stratification (Sandhu and Conev, 2014). Post-cold stratification seeds need to be warmed in order for the embryo to mature and reduce the seed coat (Chen et al., 2008). For example, in a study by Zlesak (2005) done in the USA, cold stratification was implemented on *R. rubiginosa* seeds for 12 weeks at 4 °C, then seeds were moved to 14 °C where germination was observed at 11 weeks in year 1 with a total of 22.8% seeds germinated, and at 16 weeks in year 2 with a total of 40.6 %. Comparatively, Zimmermann et al. (2012) in Central and Southern Argentina, showed *R. rubiginosa* seeds took approximately half a year to germinate after being placed in a growth chamber in filter paper and moistened with deionized water, and germination rates were low (≥ 14 - 49%). Radoglou (2015) in Xiloupoli combined pericarp scarification (15 min of sulfuric acid) with warm (four

weeks) and cold (20 weeks) stratification and achieved 38 % germination. The only known attempt on plant populations occurring in the Southern hemisphere was by Mokhobo (2018), who attempted various combinations including soaking *R. rubiginosa* seeds with hot water for 5 minutes and treating them with concentrated sulphuric acid for 60 seconds. However, no seeds germinated. This current study agreed with most of the studies that *R. rubiginosa* needs cold stratification to germinate, as observed by seeds from the cold stratification treatment being the only ones that germinated, although the germination percentage was low at only 1.25%. It is not known if seeds in Southern Africa could have a lower germination rate than in the USA and South America. However, the spreading populations of *R. rubiginosa* suggest that even though germination rates might be low, sufficient seeds are germinating for the invasive population to continue spreading.

2.4.2. Seed viability/longevity

To determine the seed persistence, seeds were buried under the soil and randomly selected subsamples were removed monthly. Burial dynamics can be influenced by rainfall, temperature, and seed weight, but since *R. rubiginosa* seeds are relatively small it can be expected that once they have fallen to the ground, they are easily incorporated into the soil (Bekker et al., 1998; Benvenuti, 2007). Small seeds tend to form more persistent seed banks than larger-seeded species (Bekker et al., 1998). The current study showed that *R. rubiginosa* seeds have a persistent seed soil bank, similar to other Rosaceae species growing in the same environment. Moloi (2022) studied *C. pannosus* which showed a high seed viability of almost 60% initially and showed no significant difference in viability after being buried for 183 days and 365 days. In contrast, Adams et al. (2023) showed *P. angustifolia* had high seed survival in the first three months after being buried but the seeds did not survive for longer than 6 months in the soil. According to Zimmermann (2012) in Central and Southern Argentina, *R. rubiginosa* seeds remained viable in the soil for over two years, suggesting their seeds take some time

before decaying. This is likely the same case in southern Africa as the seed viability percentage of 83% was observed for *R. rubiginosa* seeds buried for 17 months.

2.4.3. Soil seed bank

The dispersal of many plant species' seeds over both short and long distances is actively promoted by mammals and birds in the terrestrial habitat (Manzano and Malo, 2006; Sekercioglu, 2012). Furthermore, rodents are known to bury seeds in the soil, which can increase germination success (Godó et al., 2022). *Rosa rubiginosa* seeds are dispersed by mammals and birds (Sithole, 2023), which also contributes to the seeds being deposited into the soil at different distances away from the shrub. This study showed decreasing seed numbers with distance from the shrub. Underneath the shrub, the colour of the seeds was both dark and light, a dark colour illustrating that they had been in the soil for some time, while the light colour showed the seeds were deposited in the soil recently. The deep dormancy of the seeds allows them to accumulate in large numbers, primarily below the parent shrub. As the distance from the shrub increased, the number of seeds decreased in the soil seed bank. The results obtained here support the observation from the field that *R. rubiginosa* seedlings are found mostly underneath their own canopy, as this study shows that a high number of seeds are found underneath the canopy. This would, in turn, suggest that *R. rubiginosa* will spread slowly outwards from a parent plant forming dense monocultures rather than from multiple new populations. This supports Westwood (2020) who investigated the impacts associated with *R. rubiginosa* and suggested that most of the impacts were associated with large dense stands impacting livestock access to rangelands.

2.4.4. Fruit and seed production

To determine how many *R. rubiginosa* fruits and seeds were produced per shrub, size measurements, and fruit counts were made at three study sites in the eastern Free State. Results showed a linear relationship, indicating that larger shrubs produced more seeds than smaller

shrubs. It was also determined that the shrubs were producing extremely high numbers of fruit and therefore seeds. While fruit numbers were lower than that of the two other species growing in the same area (*P. angustifolia*, Adams et al., (2023) and *C. pannosus*, Moloi (2022)), the high seed numbers per fruit, and therefore total seed production, suggest that the number of seeds entering the environment is, however, similar to the other Rosaceae species.

2.4.5. Seed predation of *Rosa rubiginosa*

A low percentage (1.28%) of *R. rubiginosa* seeds were predated by *M. aculeatus*, in this study. The first observation of wasps emerging was in September and the last was in October. Adult *M. aculeatus* females oviposit when the fruit development begins just after petal fall, and *M. aculeatus* offspring overwinter as mature larvae within the seed, pupate, feed on the seed, and emerge as adults (Rouco and Norbury, 2013).

The study revealed the alien wasp *M. aculeatus* was the only endophagous seed predator of *R. rubiginosa* in the eastern Free State Province. *Rosa rubiginosa* seeds and fruits are consumed by vertebrates (e.g., rodents, birds) (Howe and Smallwood, 1982). The wasp was found at all sampled locations, however further sampling across the entire range of *R. rubiginosa* in SA would be required to determine its distribution. The seed parasitoid *M. aculeatus* was introduced in SA due to the high number of *R. rubiginosa* seeds in the environment, but since it only damages a low percentage of the seeds, it is probably not a strong limiting factor to the spread of *R. rubiginosa*. From the perspective of *R. rubiginosa* being a damaging invasive species, the low rate of seed predation in SA is a cause for concern, as the wasp could have been considered a potential biological control agent. Therefore, determining the extent to which seed predation reduces the invasive potential of *R. rubiginosa* is important to know if it could contribute to reducing its further spread (Milton et al., 2007; Iponga et al., 2008). The low levels of damage in SA suggest that there is little impact from the wasp and that the plant is likely to increase in density and distribution via seeds in the absence of further management. It

is therefore suggested that biological control be considered for *R. rubiginosa* in SA and that seed-feeding agents should be considered despite the presence of the seed-attacking wasp.

2.5. Conclusion

Rosa rubiginosa shrubs produce many fruits depending on the shrub volume, and their fruits consist of many seeds. Seeds are deposited in high numbers directly underneath their canopy. However, the *R. rubiginosa* seeds have deep dormancy which prevents frequent germination, and their seeds remain in the soil for a long period of time. It appears that the seeds require some cold stratification to germinate, although germination success is low, even with cold stratification. . This shrub has a seed predator *M. aculeatus* which has been shown to have less of an effect on damaging *R. rubiginosa* seeds than expected.

Chapter 3

Impact of *Rosa rubiginosa* on native and invasive woody species in grasslands of the eastern Free State

3.1. Introduction

Grasslands, including open grassland, grassy shrublands, and savannas, cover about 40% of the Earth's surface and 69% of Earth's agricultural land area (Suttie et al., 2005; O'Mara, 2012). Many grasslands are known as biodiversity hotspots due to their high plant diversity (Gibon, 2005; Reidsma et al., 2006). Grasslands also provide a wide range of ecosystem services such as food production, recreation, tourism, biodiversity maintenance (Duelli and Obrist, 2003; Bonari et al., 2017), and goods and services that support human populations worldwide (White et al., 2000). Furthermore, the degradation of grasslands is widespread and accelerating in many parts of the world (White et al., 2000), with 49% of grassland areas globally having been degraded to some extent (Gang et al., 2014; Gibbs and Salmon, 2015). In South Africa (SA), grasslands are highly transformed, poorly conserved, and in desperate need of restoration (Reyer et al., 2005; Carbutt and Martindale, 2014). One of the problems in grasslands is invasive species transforming landscapes and promoting the spread of other invasive species, reducing native plant diversity (Lambert et al., 2010). This invasion by alien plants is a growing challenge worldwide for the management of native biodiversity and ecosystem functioning of grasslands (Meyer, 2014).

Most woody invasive alien species in the grasslands of SA, particularly in the genera *Acacia*, *Hakea*, and *Pinus*, form dense stands or monocultures that significantly impact ecosystems (Versfeld and Van Wilgen, 1986). Some invasive species are known to facilitate species richness (Callaway, 1995; Callaway and Walker, 1997). Unfortunately, this facilitation is often of other alien species (Callaway, 1995; Callaway and Walker, 1997; Zélé et al., 2018), that may have severe impacts. Facilitation can take many forms, for example, in the protection of

seedlings. The protection of seedlings by larger plants is called the "nurse plant syndrome" (Niering et al., 1963). Nurse plants facilitate the growth and development of other plant species found under their canopy, by offering benign microhabitats that are more favourable for seed germination and/or seedling recruitment than their surrounding environment (Ren et al., 2008). Seedlings may be protected by nurse plants from herbivory damage (such as grazing and trampling; Bonanomi et al., 2011), desiccation (Gómez-Aparicio et al., 2004), fire (Pausas and Keeley, 2014; Pistón et al., 2016), or even increasing water (by decreasing evaporation) and nutrient availability through the litter of nurse plants (Nobel and Zutt, 2005; Padilla and Pugnaire, 2006; Ren et al., 2008).

The protection of seedlings is usually achieved through specific attributes of "nurse plants", for example, they may be unpalatable (Smit et al., 2006; Bee et al., 2009), form dense impenetrable stands, or be thorny to prevent grazing animals from accessing seedlings (Flores and Jurado, 2003; Callaway, 2007; Smit et al., 2007; Smit and Ruifrok, 2011). Some species may alter soil characteristics chemically or mechanically which may favour the development of seedlings (Long et al., 2015). Finally, nurse plants offer perching points to bird species which allows for the accumulation of seeds below the canopy of the shrub (Verdú and García-Fayos, 1998).

Several Rosaceae species (primarily alien) are forming dense community complexes in the grasslands of SA (Figure 3.1). The dominant shrubby species within these invasive Rosaceae complexes are *Cotoneaster pannosus* Franch., *Pyracantha angustifolia* (Franch.) C.K. Schneid., *Rosa rubiginosa* L., and the indigenous woody encroacher *Leucosidea sericea* Eckl. & Zeyh. Whether these species are facilitating woody invasions into South African grasslands is currently undetermined. However, in the mountain grasslands of Argentina, Tecco et al. (2006) showed a high richness of both native and exotic woody plant species (WPS) underneath

canopies of the invasive thorny Rosaceae shrub *P. angustifolia* compared to under canopies of the native shrub *Condalia montana* A.Cast. (Rhamnaceae) canopies or open vegetation, suggesting that *P. angustifolia* was acting as a nurse plant. Similarly in Argentina (Patagonia), Svriz et al. (2013), showed that native forest species recruitment occurred only under deciduous *R. rubiginosa* when compared to open grassland sites.

Figure 3.1: A Rosaceae species complex found in the grassland biome in South Africa. Orange fruiting species: *Pyracantha angustifolia*; red fruiting species: *Cotoneaster pannosus*; light green leaved species: *Leucosidea sericea*; and despite not being clear, *Rosa rubiginosa* is also present in the image, mostly overshadowed by the taller Rosaceae species. Photo credit: G.D. Martin.

3.2. Aims and objectives

This study aimed to determine if *R. rubiginosa* is acting as a nurse plant and facilitating recruitment, by assessing the difference in abundance and diversity of native and invasive woody plant species under the canopies of two woody Rosaceae species: *R. rubiginosa* (invasive) and *L. sericea* (native), and adjacent open grassland (control) at three sites. This

study also aimed to determine if the size of the shrub influences the number of plant species found under it.

3.3. Materials and methods

3.3.1. Study sites

The study sites are located in the foothills of the Northern Maloti Drakensberg near the town of Clarens, eastern Free State province, SA. The sites are privately owned farms, namely Clifton farm sites 1 and 2 (S 28°32.844, E 28°25.215; S 28°54.067, E 28°42.927; respectively), and Rockhill farm (S 28°29.003, E 28°49.443). Different experiments within this study were conducted at various points between September 2021 and March 2023.

The mean annual temperature for Clarens is typically 13 °C, varying from a mean minimum of 1.8 °C to a mean maximum of 27.2 °C (Moffett, 2018; Figure 3.2), with frost reducing the temperature to well below freezing. Most precipitation is in the form of rain, almost all falling from November to March (Moffett, 2018).

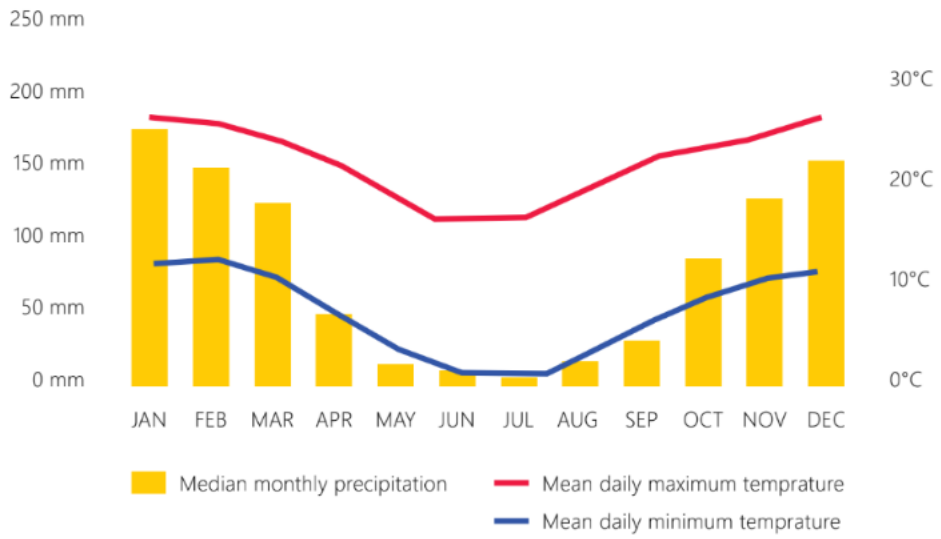

Figure 3.2: Climate (minimum and maximum daily temperatures, and monthly rainfall) for the Northern Drakensberg Highland grassland vegetation group, which broadly reflects the climate of the Clarens Conservancy (graph extracted from Moffett, 2018).

The sites studied fall into The Drakensberg Grassland which consists of two main vegetation types (Gd5 and Gd7) which harbour more than 60 grass species (Rutherford et al., 2006). Three of the most common grass species found here are blue love grass (*Eragrostis chloromelas* Steud.), red grass (*Themeda triandra* Forssk.), and thatch grass (*Hyparrhenia hirta* (L.) Stapf) (Moffett, 1997). These study sites are heavily invaded by alien Rosaceae species and are also being encroached by the native WPS, *L. sericea*. This study focused on investigating *R. rubiginosa* as a potential nurse plant for other invasive species, in comparison to the native *L. sericea*. *Rosa rubiginosa* is native to Eurasia and is transforming high-altitude grasslands in the eastern Free State into impenetrable thorny thickets. Similarly, the native *L. sericea* is a woody encroacher in these montane systems.

Leucosidea sericea, commonly known as oldwood (ouhout), is only found in southern Africa, where it occurs abundantly in SA and Lesotho and to a lesser extent in Eswatini and Zimbabwe (Mafole et al., 2017). It is an evergreen shrub or tree with a height ranging from 2 - 9 m (Buzunova et al., 2011; Pitso and Ashafa, 2015; Figure 3.3). It is highly adapted to a wide range of habitats including grasslands, riverbanks, rocky ridges, damp deep sandy or clayey and rocky soils (Pooley, 1993). This species forms dense thickets in overgrazed and disturbed areas and often exhibits a tendency to become an aggressive invader on farmlands (Pond et al., 2002; Daemane et al., 2010).

Figure 3.3: Clifton farm (site 2) where *Rosa rubiginosa* (white arrow) and *Leucosidea sericea* (black arrow) are found in abundance. Photo credit: G.D. Martin.

3.3.2. Recruitment richness and density

Using modified methods from Tecco et al. (2006), the number of all WPS was counted in three 'treatments' at each site: under 20 *R. rubiginosa* canopies, under 20 *L. sericea* canopies, and in ten 16 m^2 plots of herbaceous grassland that forms the matrix between woody shrubs at the study sites (i.e. with no shrub cover; a total of 50 samples for each site). Shrubs of both species and plots were chosen randomly at each experimental event within each of the three sites. The number and identity of WPS of a height of 10 cm and above were determined for each canopy/plot. Representative specimens of each species were collected and deposited at the Qwaqwa Herbarium at the University of the Free State (Figure 3.4). In addition, the height and widths at two points (North-South and East-West) of each of the focal shrubs were measured at their widest points to allow for the calculations of the area under the canopies and the volume of the shrubs. To assess the relationship between the observed sapling richness, density, and/or the size of the shrub canopies, the canopy cover for each focal shrub was estimated by calculating the area as $\pi \times r^2$, where r represents the radius of the shrub in meters (m). Species

richness and density under each focal species were modelled using Generalised Linear Mixed Effects Models (GLMM) with a Poisson distribution and log link function. Data were not normally distributed (Shapiro Wilks Normality Test, $P < 0.001$). Species richness and species density were set as target/response variables, focal species (*L. sericea*, *R. rubiginosa*, control), seedling status (native or invasive), focal shrub size (in m^3), and their interactions were set as fixed predictor variables, and the site was included as a random effect. Normality tests and GLMMs were conducted in IBM SPSS Statistics version 29.

Figure 3.4: Identifying, collecting, and counting the woody species found under the canopy of a *Rosa rubiginosa* shrub. Photo credit: K.T Moloi.

3.3.3. Temperature and Light

In addition, to determine if *R. rubiginosa* is creating a unique microclimate, HOBO MX2202 temperature and light loggers were installed. Due to limited availability of equipment, only one logger was installed below a *R. rubiginosa* canopy and one in an uninvaded plot. The loggers were programmed to record at 30-minute intervals, giving 48 readings of each parameter per

day. The data were downloaded using a Bluetooth-linked HOBO mobile application. Daily temperature and light intensity were compared between the *R. rubiginosa* canopy and open control, with normally distributed data, using a paired student's t-test and graphed using statistical analysis in R (R Core Team, 2021).

3.4. Results

3.4.1. Recruitment richness

This study showed that *R. rubiginosa* has a significant nurse plant effect on a greater number of woody plant species compared to both *L. sericea* and natural grassland ($F_{1, 351} = 8.9$, $P = 0.003$; Figure 3.5). There was no difference in the number of species recorded under *L. sericea* and in control sites ($P > 0.05$). There were significantly more native species than invasive species under all three treatments and a significant difference in the number of native and invasive species between the three treatments (Figure 3.5). No significant effect of site on species richness was evident for any treatment. Shrub size affects the number of plant species recorded underneath each focal species with larger shrubs having more WPS underneath them overall but with no difference in the number of invasive or native species found underneath focal species. On average *R. rubiginosa* had a larger shrub volume than *L. sericea*. Furthermore, across all the three sites, *R. rubiginosa* had more invasive species recorded underneath it (13 species) compared to both *L. sericea* (11 species) and the control plots (8 species). There were no differences in the number of invasive species underneath *L. sericea* and control plots. *Rosa rubiginosa* had more native species underneath it (21 species) compared to both *L. sericea* (20 species) and the control plots (13 species).

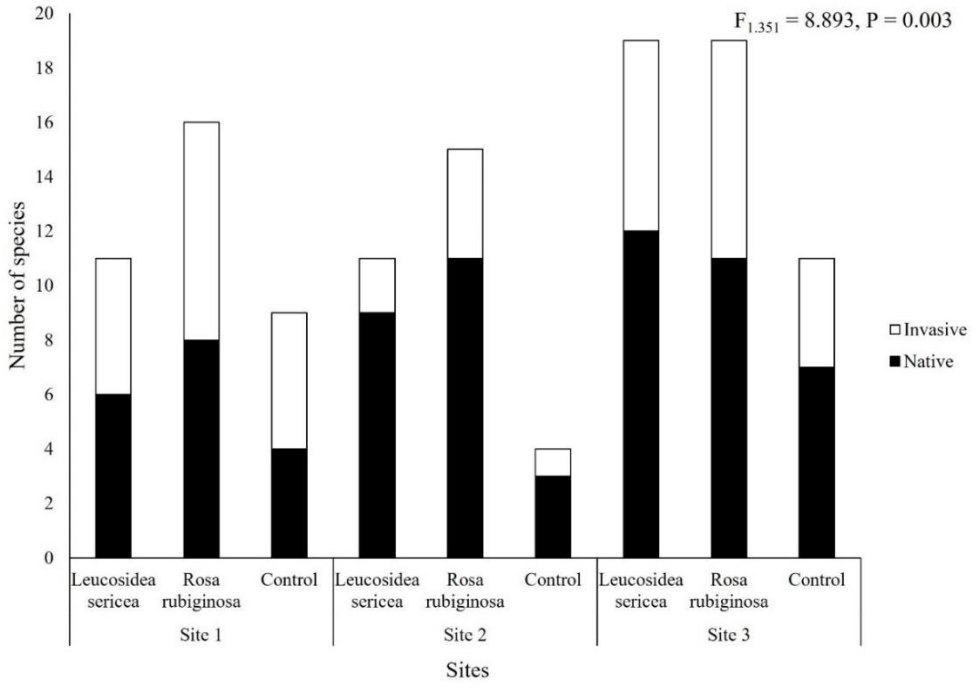

Figure 3.5: Total richness of native and invasive species (number of species recorded) under *Leucosidea sericea*, *Rosa rubiginosa*, and control (no shrub cover) at Clifton farm site 1 and 2, Rockhill farm in Clarens (eastern Free State).

3.4.2. Recruitment density

Rosa rubiginosa had a greater nurse plant effect on plant density, compared to both *L. sericea* and control sites ($F_{1, 351}$ = 45.3, P < 0.001; Figure 3.6). There was no difference in the plant density under *L. sericea* and in the control treatments (P = 0.241). The density of native and invasive species was significantly different under all three treatments ($F_{1, 351}$ = 5.5, P = 0.019), and there was a significant difference in the density of native and invasive species among the three treatments ($F_{2,351}$ = 30.8, P < 0.001). There was no significant effect of the site (P = 0.396). Shrub size affected the number of plant species recorded underneath each focal species ($F_{1, 351}$ =7.3, P = 0.007), with larger shrubs having a greater density of plant species overall, and larger *R. rubiginosa* shrubs tended to have a significantly higher density of plants under their canopies ($F_{2, 351}$ =4.2, P = 0.04). However, there was no effect of shrub size on the density of invasive or

native species found underneath a focal species ($F_{1, 351} = 0.2$, P =0.677). *Rosa rubiginosa* had a greater density of invasive species (0.3 m^2) underneath it compared to both *L. sericea* (0.0 m^2) and the control plots (0.1 m^2; Figure 3.6). Furthermore, there was no difference in the density of invasive species underneath *L. sericea* and control plots, with no difference in the density of native species under all three treatments (P = 0.457).

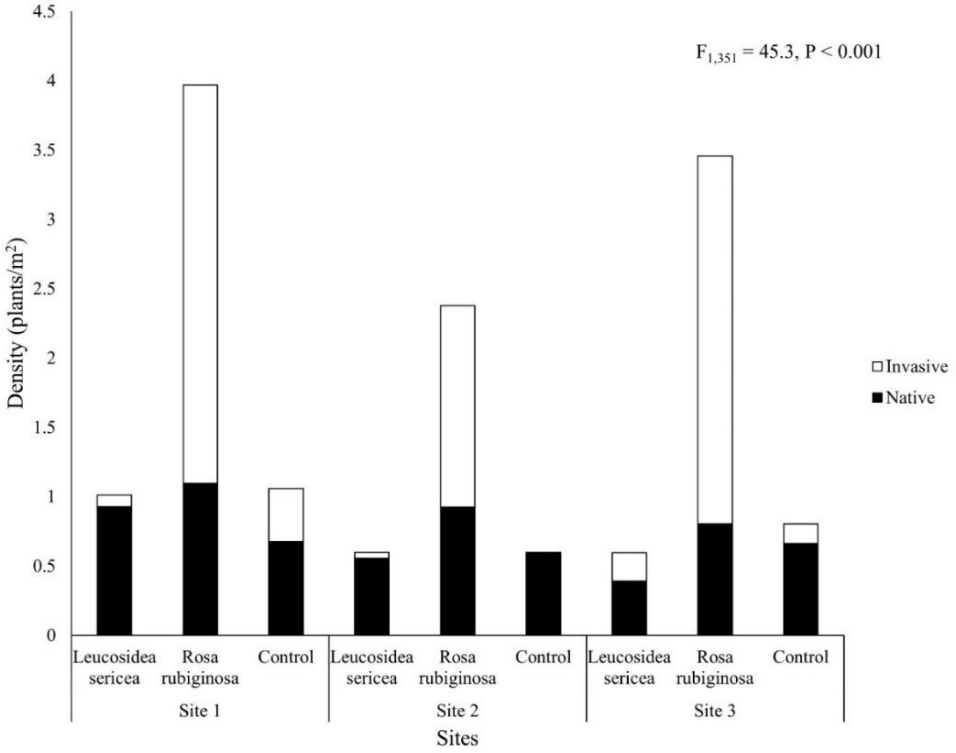

Figure 3.6: Total density of native and invasive species (plants/m^2) under *Leucosidea sericea*, *Rosa rubiginosa*, and control (no shrub cover) at Clifton farm site 1 and 2, Rockhill farm in Clarens (eastern Free State).

The native species that were most frequently found in these three treatments in the studied sites was *Artemisia afra* Jacq. ex Willd. (Asteraceae), *Helichrysum cymosum* (L.) D. Don. (Asteraceae), and *L. sericea* (Table 3.1). Frequent invasive species were *Bidens pilosa* L. (Asteraceae), *R. rubiginosa* and *Rubus ulmifolius* Schott. (Rosaceae; Table 3.1). Also, mature *R. rubiginosa* shrubs nurse more of their own seedlings than any other WPS underneath its canopy.

Table 3.1: Native and invasive species observed under *Leucosidea sericea*, *Rosa rubiginosa*, and in open grassland control plots (no shrub cover) at Clifton farm sites 1 and 2, and Rockhill farm. under the tree species.

Scientific name	**Family**	**Status**	***Rosa rubiginosa* canopy**	***Leucosidea sericea* canopy**	**Control (no shrub cover)**
Agrimonia procera Wallr.	Rosaceae	Invasive	×	×	
Arctotis arctotoides (L.f.) O.Hoffm.	Asteraceae	Invasive	×	×	×
Artemisia afra Jacq. ex Willd.	Asteraceae	Native	×	×	
Asclepias gibba (E.Mey.) Schltr.	Apocynaceae	Native	×		
Asparagus officinalis L.	Asparagaceae	Invasive	×	×	
Berkheya onopordifolia (DC.) O. Hoffm. ex Burtt Davy	Asteraceae	Native	×	×	×
Bidens pilosa L.	Asteraceae	Invasive	×	×	×
Cheilanthes sp.	Pteridaceae	Native	×	×	
Cineraria dieterlenii E.Phillips.	Asteraceae	Native	×		×
Clutia natalensis Bernh.	Euphorbiaceae	Native	×	×	
Cynoglossum lanceolatum Forssk.	Boraginaceae	Native		×	×

Scientific name	Family	Status	*Rosa rubiginosa* canopy	*Leucosidea sericea* canopy	Control (no shrub cover)
Diospyros austro-africana var. rubriflora.	Ebenaceae	Native	×	×	
Felicia filifolia subsp. Filifolia	Asteraceae	Native	×		×
Felicia mossamedensis (Hiern) Mendonça	Asteraceae	Native	×	×	×
Gleditsia triacanthos L.	Fabaceae	Invasive	×		
Gomphocarpus fruticosus (L.) W.T.Aiton	Apocynaceae	Native		×	
Helichrysum aureonitens Sch.Bip.	Asteraceae	Native	×	×	×
Helichrysum cymosum (L.) D.Don	Asteraceae	Native	×	×	×
Helichrysum nudifolium var. pilosellum	Asteraceae	Native	×		×
Hermannia geniculate L.	Malvaceae	Native		×	
Heteromorpha arborescens var. abyssinica (Hochst. ex A.Rich.) H.Wolff	Apiaceae	Native	×		
Nemesia denticulate (Benth.) A.L.Grant ex Fourc.	Scrophulariaceae	Native	×	×	×
Oenothera rosea L'Hér. ex Aiton	Onagraceae	Invasive	×	×	×
Prunus persiea (L.) Batsch.	Rosaceae	Invasive	×		
Pyracantha angustifolia (Franch.) C.K. Schneid.	Rosaceae	Invasive	×	×	×

Scientific name	Family	Status	*Rosa rubiginosa* canopy	*Leucosidea sericea* canopy	Control (no shrub cover)
Rhammus prinoides L'Hér.	Rhamnaceae	Native	×	×	
Robinia pseudoacacia L.	Fabaceae	Invasive		×	
Rubus ludwigii Eckl. & Zeyh.	Rosaceae	Native	×		
Rubus ulmifolius Schott.	Rosaceae	Invasive	×	×	×
Salvia repens Burch. ex Benth.	Lamiaceae	Native	×	×	×
Searsia dentata (Thunb.) F.A.Barkley	Anacardiaceae	Native	×	×	
Searsia discolor (E.Mey. ex Sond.) Moffett	Anacardiaceae	Native	×	×	
Senecio rhomboideus Harv.	Asteraceae	Native	×	×	
Solanum incanum L.	Solanaceae	Native			×
Tagetes minuta L.	Asteraceae	Invasive	×	×	×
Verbena bonariensis L.	Verbenaceae	Invasive	×		×

3.4.3. Temperature and Light

The average temperature per day was higher in the open grassland (17.7 °C) compared to the *R. rubiginosa* understory (14.6 °C; Figure 3.7). Similarly, the average light intensity was higher in open grassland (17544.4 lux) than under a *R. rubiginosa* canopy (3940.5 lux; Figure 3.8). Higher temperatures and light intensity were observed in summer (Open grassland: 18.48 °C, 4767.81 lux; *R. rubiginosa* canopies: 21.96 °C, 24670.51 lux; respectively), and lower temperatures and light intensity were observed in winter (Open grassland: 9.08 °C, 15639.92 lux; *R. rubiginosa* canopies: 10.40 °C, 3458.0 lux; respectively). The daily average temperature and light intensities were significantly different in open grassland and underneath *R. rubiginosa* shrubs (Temperature: $t = 27.1$; $df = 43554$; $p < 0.05$) (Light: $t = -69.6$; $df = 43554$; $p < 0.05$).

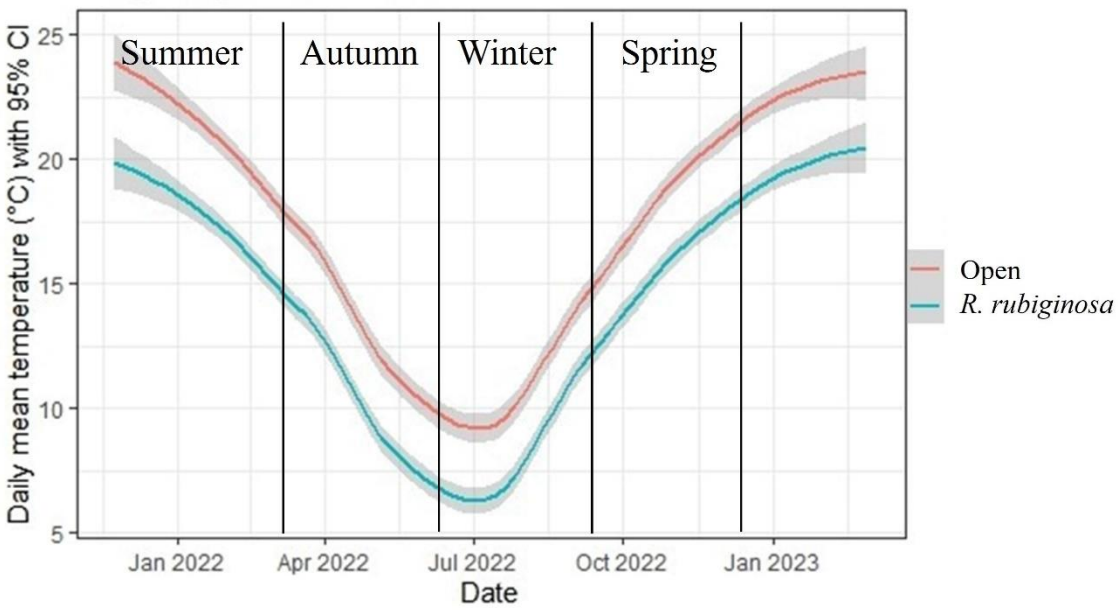

Figure 3.7: Daily mean seasonal temperatures under *Rosa rubiginosa* and in open grassland in the eastern Free State, South Africa. Shaded areas show confidence intervals.

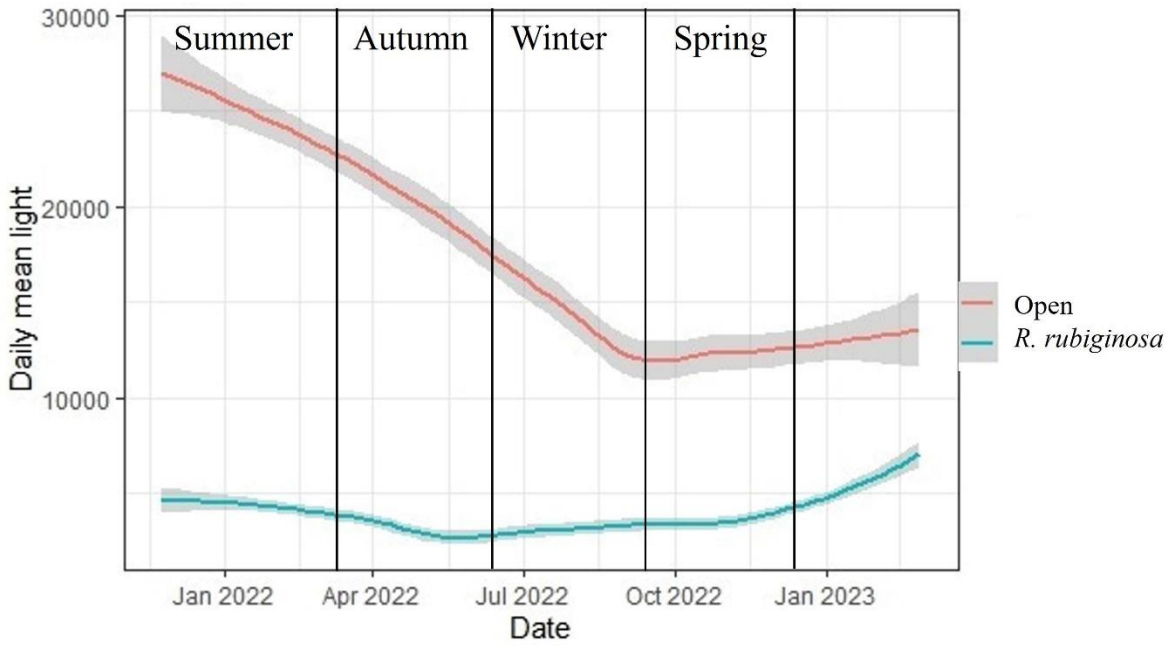

Figure 3.8: Daily mean seasonal light intensity under *Rosa rubiginosa* and in open grassland in the eastern Free State, South Africa. Shaded areas show confidence intervals.

3.5. Discussion

Positive facilitation between plants has been recognised as a factor shaping plant communities (Callaway, 1995). This study showed that *R. rubiginosa* acts as a nurse plant, to both native and invasive plant species. Moreover, a greater number of different species were found at greater densities underneath the canopies of *R. rubiginosa*, compared to *L. sericea* and open grassland control plots. Although *L. sericea* acts as a nurse plant, it is to a lesser extent than *R. rubiginosa* and rather is similar to open plots. Specifically, *R. rubiginosa* appears to be a significant nurse plant for other invasive species. Finally, larger shrubs, especially *R. rubiginosa*, tends to have more WPS at greater densities than both *L. sericea* and control open plots.

Rosa rubiginosa is a dense deciduous shrub, which creates a cool, dark microhabitat underneath it's canopy, where compared to open grassland. Furthermore, the stems bear numerous curved,

hooked thorns and bristles (Aguirre et al., 2009; Gadzinowska et al., 2019). These thorns act as mechanical protection against browsing by large mammals (Bruno et al., 2003; García and Obeso, 2003) but also as protection for smaller mammals such as rats and mice who may act as seed and seedling predators (Sage et al., 2019; Morris et al., 2022). This suggests that not only does *R. rubiginosa* protect some plant species once established but it may also protect seed predators which may target seeds. In addition, it creates favourable micro-environmental conditions which benefit seedlings (Callaway, 1992; Kitzberger et al., 2000). There are significant reductions in light intensity underneath the canopy that would favour seedling growth but also temperature which would decrease evaporation providing more moisture for developing seedlings (Clinton, 2003; Carlson et al., 2020). The grasslands of SA are among the most threatened ecosystems and unfortunately also one of the least conserved (Clark et al., 2022). They are facing several threats including, habitat change, climate change, changing fire regimes, and bush encroachment from both native and alien species (SANBI, 2013).

Rosa rubiginosa is a hardy plant that can acclimate to dry habitats and soils poor in nutrients or with high salinity levels (Robert et al., 2003; Hura et al., 2017). It has been suggested by Hura et al. (2017) that this adaptability could contribute to its effective functioning as both a nurse and pioneer plant, particularly in the hot and dry southern hemisphere. A study by Hura et al. (2023) in a fire-affected area of Nahuel Huapi National Park in Patagonia, recorded *R. rubiginosa* shrubs blooming and bearing fruit only 2 years after a fire. Furthermore, due to inherent phenotypic plasticity and ecological flexibility, *R. rubiginosa* also has a high potential to colonize degraded and disturbed areas (Zimmermann et al., 2014) while is also capable of being resistant to hostile conditions (e.g., drought, frost; Pierce et al., 2017). *Rosa rubiginosa* can re-sprout after cutting or fire (Smulders et al., 2011). *Rosa rubiginosa* is unpalatable, protecting saplings underneath its canopy against browsing cattle and increasing sapling survival (Smit et al., 2006; 2007), while native *L. sericea* are palatable and widely browsed by

livestock during spring (Pooley, 1993). This may explain why more seedlings of *R. rubiginosa* were located below adult *R. rubiginosa* plants than below *L. sericea*.

The successful recruitment of species is influenced by the phenology of seed dispersers, and the timing in which they disperse the seed (Jordano, 2000). *Rosa rubiginosa* seeds serve as food for rabbits and rodents, and the passage of seeds through the digestive tract does not diminish their viability but accelerates their invasion success (Traveset et al., 2001; Bobadilla et al., 2020; Godó et al., 2022). Rodents may also carry *R. rubiginosa* seeds to their burrows/nests under the soil and in rock crevices which can help seeds that survive predation to stay viable and later germinate (Godó et al., 2022). Furthermore, a study by Sithole (2023) using remote cameras triggered by motion, showed that rodents and birds frequently carry away fruits of *R. rubiginosa* from under these shrubs, which also contributes to their spread.

3.6. Conclusion

This study showed there were significantly more native species than invasive species under all three treatments. Shrub size affects the number of plant species recorded underneath each focal species with larger shrubs having more WPS underneath them overall. Across all the three sites *R. rubiginosa* had more invasive and native species recorded underneath it compared to both *L. sericea* and the control plots. The average temperature and light per day showed to be higher in the open grassland compared to the *R. rubiginosa* understory, suggesting that *R. rubiginosa* creates a darker, cooler microclimate, which may be ideal for recruitment. *Rosa rubiginosa* species showed to significantly facilitate the establishment of WPS, particularly invasive species, possibly further contributing to bush encroachment. Due to the abundance, wide distribution, and rapid spread of *R. rubiginosa* in SA, this is very concerning and should be considered in national weed management plans. However, the severe potential implications of

spreading invasive species and the woodification of one of SA's most important biomes should take priority.

Chapter 4

General discussion

4.1. Introduction

The aim of this study was to investigate various aspects of the reproductive ecology of the invasive alien species *R. rubiginosa* in the eastern Free State Province of South Africa (SA) and to assess if this species is facilitating the spread of other woody encroaching species in the montane grasslands of the Maloti-Drakensberg mountains. The objectives included investigating the seed ecology of *R. rubiginosa* by determining what contributes to its germination, the number of seeds produced by plants, the seeds' life span in the soil, and determining the impact that *Megastigmus aculeatus* has on *R. rubiginosa* seeds (Chapter 2). Finally, the study aimed to determine whether *R. rubiginosa* facilitates woody invasions into the grassland (Chapter 3). In this chapter (chapter 4), conclusions, management implications, and recommendations are provided.

4.2. General findings

This study showed that *R. rubiginosa*, under these experimental conditions, has a low germination percentage of 1.25%. Only seeds that were refrigerated (exposed to extensive cold stratification) germinated, while all seeds collected monthly from the field (natural cold stratification) failed to germinate. In addition, there was delayed germination since the first germinated seed was only observed after nine months. While conducting experiments in the field, low numbers of seedlings were observed supporting these results and suggesting that even in the field germination rates are low. However other biotic factors such as rodent seed predation and seedling herbivory may also be reducing seedling development and require further investigation (Mokotjomela, 2016; Morris et al., 2022).

In addition, to the suggested impacts of rodents, we suspected that seed predator *M. aculeatus* may also be having an impact on the availability of viable seeds. Results showed that a total of 148 *M. aculeatus* emerged from 700 fruits collected, giving an overall percentage of 1.28% of seeds predated (Chapter 2). The highest number of *M. aculeatus* emerged from the seeds collected in June and July 2021, with no emergence in April, May, and August 2021. This showed that, currently, *M. aculeatus* probably does not have a significant impact on the overall amount of viable *R. rubiginosa* seeds entering the environment. Unless mass reared, *M. aculeatu*s should not be considered a biocontrol agent due to this low predation rate. For a seed-damaging biological control agent to be effective, a large ecosystem-level impact of up to 98% of seeds being damaged is needed (Hoffmann and Moran, 1991; Salgado and Martin, 2023).

Megastignus aculeatus was present in 1.28% of seeds, despite not reducing the spread of the plant, and considering the high numbers of fruit produced each season by these plants, millions of *M. aculeatus* are entering the environment every year. It is worth remembering *M. aculeatus* is also an introduced species in South Africa and is known to be the generalist seed predator of Rose species (Doğanlar, 2015). It thus requires further investigation and should be treated as a potentially damaging invasive alien species. The limited number of months the wasp is associated with *R. rubiginosa* seeds also suggests that it is likely using other hosts in the grassland biome. These other host species were not identified in this study, but other Rose species should be investigated as potential host plants, as the wasp may be having a significant impact on native species or other commercial Rose species.

Rosa rubiginosa acts as a nurse plant for both native and invasive woody seedlings, with more species recorded under *R. rubiginosa* compared to the native *Leucosidea sericea* and an open grassland control. The canopy of *R. rubiginosa* creates a cool, dark microclimate, as shown by the cooler temperatures and decreased light intensity under *R. rubiginosa* canopies compared

to the open grassland. This may be an ideal microhabitat for recruitment. Furthermore, the thorny branches likely provide seeds and seedlings with an extra benefit of protection from seed predators and herbivores. This nurse plant effect may be facilitating the encroachment of woody species, particularly of invasive species, into the grasslands. The threat of alien woody invasions to the grassland biome of SA poses significant challenges to ecosystem health and the economy (Van Wilgen et al., 2008; McDougall et al., 2011). For example, these invasions can lead to a significant loss of biodiversity such as native plant and animal species being displaced or excluded by the invading woody plants (McNeely et al., 2001). This can disrupt the balance of ecosystems and lead to a decrease in biodiversity, which can influence the entire ecosystem (Dukes and Mooney, 2004; Ehrenfeld, 2010). The invasion of alien woody plants can also lead to a reduction in water flow in the grassland biome since woody plants have different water requirements compared to grasses (Le Maitre et al., 2015). Grasses are typically drought-tolerant and can survive in conditions of low water availability, while woody plants require more water to survive (Gorgens and Van Wilgen, 2004; Le Maitre et al., 2016). As a result, the invasion of woody plants can lead to a decrease in water availability for other species in the ecosystem, which can further worsen the loss of biodiversity (Levine et al., 2003; Cao et al., 2009). Furthermore, the invasion of alien woody plants can lead to changes in ecosystem functioning. For example, woody plants can outcompete native grasses for sunlight (see Chapter 3), leading to a decrease in the amount of sunlight available for other species. This can disrupt the food web in the ecosystem and lead to a decrease in the overall productivity of the ecosystem. The threat of *R. rubiginosa* invasions to the grassland biome of SA is a significant concern. Overall, it poses a threat to biodiversity, reduces water flow, and changes the functioning of ecosystems. Therefore, it is crucial to implement effective strategies to control and prevent alien woody invasions to mitigate these threats.

4.3. Conflict of interest species

The introduction of many invasive species is to provide food and raw materials, recreational ornamentation, erosion control, and dune stabilisation, although there were many other species introduced accidentally (Richardson et al., 2003). Most invasive species are studied for their negative impacts and not for their beneficial characteristics (Westwood, 2020). Negative impacts can include a loss of biodiversity, changes to ecosystem functioning, economic losses, or impacts on human health (Holmes et al., 2009; Powell et al., 2013; Hulme, 2014). As mentioned previously very little work has been conducted on the study species as a beneficial or damaging invasive species. This is problematic due to the rapidly growing global demand for *R. rubiginosa* oil produced by the seeds for the cosmetic industry, particularly in SA (Westwood, 2020). There are growing calls to stop management of the species from being implemented, and for companies and landowners to get permission to grow the species commercially (G.D. Martin, pers. comm.). Currently, *R. rubiginosa* is categorised as a 1b invasive species under the NEM:BA (2004) regulations meaning cultivation is prohibited; however, due to the request from industry there has been a formal request by the Department of Agriculture to SANBI to re-evaluate the species. Therefore, how the species is managed requires careful consideration. For example, there is a need to keep rural livelihoods at heart but still reduce the weed where it is having impacts and protect the grassland biome while still allowing it to significantly contribute to rural livelihoods. These kinds of decisions are greatly facilitated if suitable and reliable information regarding the species' potential as a conflict of interest species is available.

4.4. Management options

To date, this is one of the first studies to empirically provide information about the *Rosa rubiginosa's* seed ecology but also its status as a driver of other biological invasions which will

contribute to the decision-making process regarding management of this species. *Rosa rubiginosa* is a category 1b invasive plant species in SA and must be controlled according to NEM: BA (2004). The first step to controlling or managing an invasive species is being able to locate the species and get access to the species. Usually when invading the open grasslands, the round shape and size of the plant, especially when flowering and fruiting make it relatively easy to locate and manage. However, a vast majority of the plants are invading mountain systems where access is particularly difficult. Furthermore, mechanical removal of the species will be difficult, as it has numerous thorny stems and their fruits have prickles. These thorns and prickles are not only exposed on the outer part of the shrub but they are also located in the inner part of the shrub.

Fire and grazing could be considered as management options. Cummings et al. (2007) stated that burned patches of grassland reduced and maintained invasive species at the young and palatable stage of growth. However, increased grazing intensity can lead to selectivity and increase the non-uniformity of grazing of the desirable and undesirable species (Firn et al., 2013). Unfortunately, in many countries (Australia, Canada, New Zealand, and USA) grazing doesn't sufficiently manage invasive species, and where livestock and grazing is a relatively new disturbance to grasslands, selective grazing pressure may facilitate opportunities for invasive species to establish (Firn et al., 2013).

Marais et al. (2004) showed the control of woody and thorny species when compared to other invasive alien species is particularly difficult due to costs and amount of biomass, especially in ecosystems prone to fire such as the grasslands of the eastern Free State. Management of *R. rubiginosa* seeds will also be difficult since their seeds stay viable in the soil for a long time, as shown by the current study. However, *R. rubiginosa* seeds need cold stratification and have slow germination rates, which may facilitate management initiatives.

4.5. Future research

During this study, *R. rubiginosa* was noticed to have slight variation amongst the shrubs that were observed, potentially suggesting either the presence of hybrids or that a potential cryptic invasion by a similar *Rosa* species such as *Rosa canina* L. may be occurring. To the best of our knowledge, however, there is no information or record of *R. canina* in SA. According to Porter (2022), *R. rubiginosa* and *R. canina* can hybridize with each other, and hybridization with our native species is unlikely as they are only distantly related. These variations observed included different flower colours: one morph had white petals and the other had pink-pale and/or pink petals fading to a white base (Figure 4.1). Literature suggests, for example, that *R. rubiginosa* flowers are predominantly pink-pale, pink with a white centre, or uniformly bright pink (Henderson, 2001; Cavallero and Raffaele, 2010; Maitra et al., 2016), while *R. canina* is commonly known as a white-flowered rose (Maitra et al. 2016). However, Kizil et al. (2018) and Phlivan et al. (2018) state that *R. canina* flowers are white and pink in colour.

Figure 4.1: *Rosa rubiginosa* flowers with different coloured flowers (pale pink on far left, white in the middle photo or pink with a white centre on far right) observed in Clarens, eastern Free State Province, South Africa. Photo credit: G.D. Martin.

The fruits colour and shape also differed for plants of our study population; some fruits were orange and roundish with many prickles on the surface, while the others were redder and oval with a smooth surface (few to no prickles) (Figure 4.2 A and B). Uggla et al. (2005) suggested *R. rubiginosa* fruits turn from orange to red, similar to Aguirre et al. (2009) who stated *R. rubiginosa* fruits were orange-red and with prickles. *Rosa rubiginosa* has rounded leaf tips while *R. canina* has tapering leaf tips (Porter, 2022). While in the field, we observed that plants with white flowers had tapering leaf tips and the plants with pale-pink flowers had rounded leaf tips (Figure 4.1). Another potential diagnostic variation was that the abaxial side of the leaves of one morph had a high trichome density whilst the other had smooth leaves (Figure 4.2 C and D). In addition, the stems were different with one morph having brownish stems while the other had green-purplish stems.

In summary, some plants had white flowers, round orange fruits with few to no prickles, and no trichomes on the abaxial side of the leaf, while other plants had pale pink flowers, red fruits without prickles, and many trichomes on the abaxial side of the leaf giving them a furry feeling. This matches a description provided by Porter (2022) who stated that "The sweetbriar (*R. rubiginosa*) and dog roses (*R. canina*) can be told apart from each other most easily because sweetbriar rose has stalked glands (hairs) on its sepals, and hairs and stalked glands underneath the leaves while dog rose does not. The leaves of sweetbriar rose have a sweet smell while dog rose leaves are not scented. Other identification traits that are useful in the inland Pacific Northwest area are the pointedness of the leaves and flower colour. Dog rose usually has pointier leaves and white flowers, while sweet briar rose has rounder leaves and pink flowers".

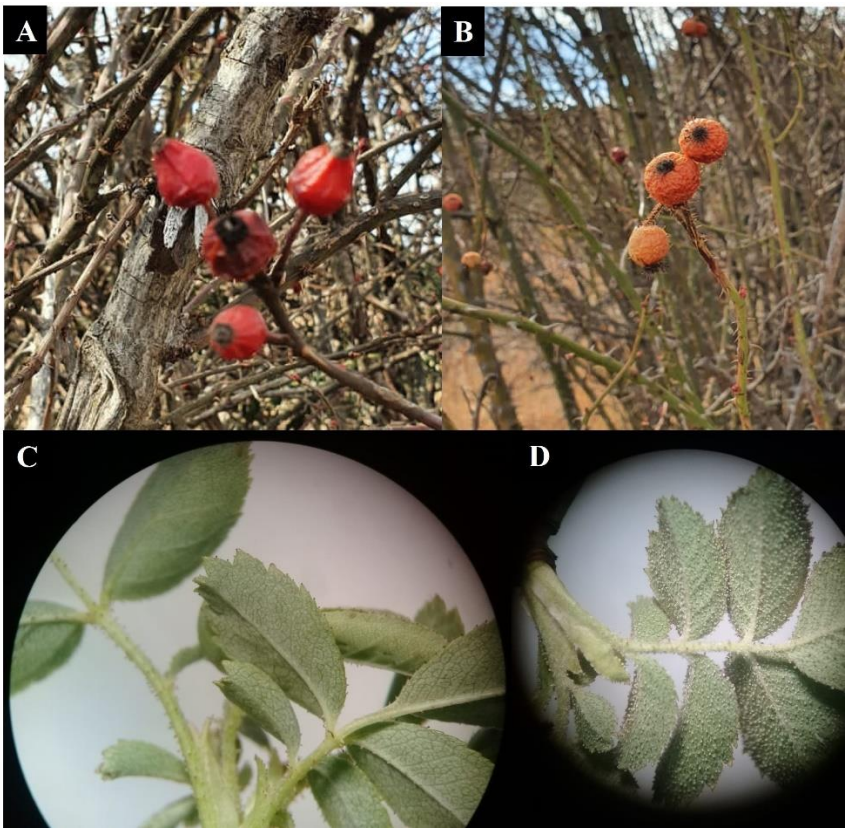

Figure 4.2: *Rosa rubiginosa* fruits: (A) red with an oval shape and (B) orange and round shape; and leaves showing a (C) smooth abaxial surface with trichomes (D) observed in Clarens, eastern Free State Province, South Africa.

Rosa rubiginosa can be quite morphologically diverse, with observed morphological differences that require a quantitative morphological study or a genetic analysis. It has been established already that, in SA, there is a lack of studies on:

- *Rosa rubiginosa* invasions, including why this species is becoming dominant in the mountainous regions compared to other countries.
- What facilitates its rapid spread outside of the Free State grasslands; we assume this to be through rodents, larger mammals, and birds but this is unconfirmed in SA.
- Low germination rates despite many seeds entering the environment, and it is undetermined if there are other mechanisms at play that may be prohibiting its spread.

- *Megastigmus aculeatus* damage to the *R. rubiginosa* seeds. While this study did investigate this to some extent, it barely scratched the surface of this relationship and the biology of the wasp. This interaction deserves further investigation.

4.6. General conclusion

Rosa rubiginosa seeds have deep dormancy preventing frequent germination, although the shrubs produce many fruits with many seeds in them. Furthermore, their seeds are deposited in high numbers underneath their own canopy and remain in the soil for a long period of time. Furthermore, *R. rubiginosa* shrubs have a seed predator, *M. aculeatus*, which has shown to have less of an effect on damaging *R. rubiginosa* seeds than expected. *Rosa rubiginosa* acts as a nurse plant to woody plant species found underneath its canopy. These plants were facilitated by *R. rubiginosa* and this study showed it was facilitating both invasive and native species. Moreover, when compared to both *L. sericea* and the control plots, *R. rubiginosa* was shown to accommodate more species, especially more native species than invasive species. This leads to the conclusion that *R. rubiginosa* contributes to bush encroachment.

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