Cape Peninsula
University of Technology

SOWING DENSITIES FOR ACTIVE RESTORATION IN SWARTLAND SHALE RENOSTERVELD VEGETATION, WESTERN CAPE

By

Thabang Makola

Thesis submitted in fulfilment of the requirements for the degree

Master of Conservation Science

in the Faculty of Applied Sciences

at the Cape Peninsula University of Technology

Supervisor: Prof Sjirk Geerts

Co-supervisor: Mr Roger Oliver

Co-supervisor: Dr Mlungele Nsikani

Cape Town

2025

SANBI
Biodiversity for Life
South African National Biodiversity Institute

Declaration

I, Thabang Makola, declare that the contents of this dissertation/thesis represent my own unaided work, and that the dissertation/thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

29 August 2025

Signed **Date**

Abstract

Ecological restoration has been recognized as a crucial strategy for mitigating habitat degradation and biodiversity loss, particularly within the Cape Floristic Region (CFR), a global biodiversity hotspot. This study focuses on active restoration of the Swartland Shale Renosterveld (SSR), a critically endangered vegetation type within the CFR, where restoration efforts are hindered by limited seed availability and low recruitment.

This thesis aimed to evaluate restoration success over time, determine optimal seed sowing densities for effective SSR restoration, and investigate species-specific limitations affecting plant establishment. Field experiments were conducted at Tygerberg Nature Reserve (TNR), where different seed sowing densities (50%, 75%, 100%, and 125% of the standard sowing density) were tested sowing seed mixes of 41 species. Restoration outcomes were assessed based on species richness, vegetation cover, and species abundance. Results show that similar restoration outcomes could be achieved using half the current standard sowing density, suggesting a more resource-efficient approach to SSR restoration.

Additionally, the study examined seed viability, germination, and the influence of smoke treatment on five selected species (*Dicerothamnus rhinocerotis*, *Podalyria sericea*, *Helichrysum dasyanthum*, *H. patulum,* and *H. teretifolium*) with poor seeding recruitment. Seed viability was found to be a major limiting factor in this study, particularly for *D. rhinocerotis*, which exhibited extremely low viability (<2%). Smoke treatment positively influenced germination of *P. sericea* and *D. rhinocerotis* but negatively impacted all *Helichrysum* species tested in this study. These findings highlight the importance of species-specific restoration approaches to optimize plant diversity and long-term restoration success in SSR.

By integrating optimized seed sowing strategies with targeted species-specific interventions, this study provides practical recommendations for enhancing SSR restoration efforts. The findings provide a framework for improving SSR restoration, informing future research and management strategies. Additionally, the findings contribute to the understanding of ecological restoration practices within Mediterranean-type ecosystems.

Keywords: Ecological restoration, Cape Floristic Region, seed sowing density, seed viability, species recruitment, restoration approaches.

Acknowledgements

I wish to thank:

My supervisors – Professor Sjirk Geerts, Dr Mlungele Nsikani and Mr Roger Oliver for providing great supervision and guidance throughout my studies.

- Dr Grea Groenewald, for assisting with statistical analysis and structuring my data and results.
- The Tygerberg Nature Reserve, reserve manager, Tshepo Mamabolo, for collaborating on my restoration project and providing study area, seeds and human resources to assist with conducting field experiments.
- The Tygerberg Nature Reserve supervisor – Marisa Langton, for giving support, and assisting with monitoring restoration plots throughout the experiment.
- The Tygerberg Nature Reserve staff members, EPWP members, students and interns, and Kirstenbosch National Botanical Garden staff members, for assisting with plots construction and monitoring.
- Interns and scholars from the Kirstenbosch National Botanical Garden, for assisting with field experiments preparations and construction.
- Sibonelo Mhlongo, Rene Brink, Onako Memeza, Siziphiwe Maka, Tarin Maile, and the Millennium Seed Bank Partnership, particularly Thembeka Malwane, for taking their time to help me with field assistance and data collection.
- The city of Cape Town – Westlake Nursery, for assisting with seed smoking treatment prior to field experiment.
- Mr Ntuthuko Mabuya, for keeping on pushing me, providing personal and academic mentorship throughout my studies and, for listening to me when I needed someone to share my academic struggles with.
- My friends, for keeping me motivated and always reminding me that we are all going to get these qualifications completed.
- My family – father, mother and brother for being patient with me, believing in me and pushing me to the greater extent towards my studies.
- The South African National Biodiversity Institute and Kirstenbosch National Botanical Garden – Joan Wrench Scholarship, for providing funding and resources for conducting my research project.

Dedication

I dedicate this thesis/dissertation to my two beautiful daughters (Remoakantse & Rearabetswe).

As you grow up, I hope you do not expect life to favour your dreams but embrace the challenges that come with achieving them. Nothing in life comes easy – it takes dedication, commitment, and passion to reach your goals. Ultimately, it is your attitude that determines where you will find yourselves.

Contents

List of figures

List of supplementary tables

Glossary

Active restoration – in this context, it is defined as reconstructive restoration, where a combination of eliminating a cause of disturbance to allow natural recovery of ecosystems and the interference of human assistance is applied to enhance ecological restoration to reintroduce the desired state of biodiversity (Atkinson & Bonser, 2020).

CFR – Cape Floristic Region

CoCT – City of Cape Town

Ecological restoration – the "process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed", and an ecosystem is considered restored "when it contains sufficient biotic and abiotic resources to continue its development without further assistance or subsidy." (Society for Ecological Restoration 2004).

GCFR – Greater Cape Floristic Region

Invasive alien plants (IAP's) – alien plant species that sustain self-replacing populations over times of reproduction phases, by producing reproductive offspring with large numbers and then wide-spreads from the introduction site (Zengeya & Wilson 2023).

Native – species that naturally occurs in a certain area or region without any form of human introduction (indigenous to South Africa).

Passive restoration – eliminating the cause of disturbance (e.g., invasive alien plants) on ecosystems to allow plant communities to restore themselves to their functional or original state (Holmes et al., 2022).

Propagation techniques – in this study, this refers to different methods which can be applied to reproduce plants vegetatively under controlled and monitored conditions.

Renosterveld – a vegetation type within the Fynbos biome, characterized by the dominance of *Dicerothamnus rhinocerotis* (Mucina & Rutherford 2006), forming a fire-prone, evergreen shrubland composed of low-growing shrubs, grasses, herbaceous perennials – primarily from the Asteraceae family – and geophytes (Rutherford et al. 2006).

Remnants – the remaining parts or patches of vegetation per vegetation type (e.g., remaining parts of the Swartland Shale Renosterveld).

Seed banks – soil seed banks or natural storage of seeds within soil.

Seed densities – weight or number of seeds used per unit size.

SSR – Swartland Shale Renosterveld.

TNR – Tygerberg Nature Reserve.

Vegetative propagation – reproducing plants using vegetative materials, i.e., leaves, shoots, truncheons, plantlets, etc, under controlled and supervised conditions. This can be through cuttings, layering or divisions.

CHAPTER ONE: General introduction and background

1.1 Introduction and background

1.1.1 Ecological restoration

Ecological restoration has become well-established with significant improvements since it was identified as an international scientific discipline to conserve nature (Aronson et al. 2020). It is one of the key global environmental topics that have been given attention by the United Nations (UN) (Nsikani et al. 2022), with the years '2021 – 2030' declared the 'Decade on Ecosystem Restoration' (UN 2019). Ecological restoration is defined as the "process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed", and an ecosystem is considered restored "when it contains sufficient biotic and abiotic resources to continue its development without further assistance or subsidy" (Society for Ecological Restoration 2004). Ecological restoration has been suggested as an ecological strategy to enhance biodiversity and biotic heterogenization within species (Holl, Luong & Brancalion 2022). This practice has been conducted following either passive restoration or active restoration, with applied nucleation practiced as an active restoration technique (Atkinson & Bonser 2020; Lehman, Esler & Holmes 2022). The restoration outcomes following these methods are often measured based on species richness, vegetation cover and abundance, as well as ecological processes (Ruwanza 2017; Holl et al. 2022; Holmes et al. 2022).

Holmes et al. (2022) has defined passive restoration as eliminating the degradation drivers (e.g., invasive alien plants and anthropogenic factors) on ecosystems to allow plant communities to restore themselves. This approach is a form of unassisted restoration. Passive restoration often yields varied recovery rates and if successful it takes decades to achieve restoration targets (Corbin & Holl 2012). On the other hand, many studies have proven that passive restoration cannot successfully reach maximum recovery of highly degraded ecosystems (e.g., Cowell 2007; Brancalion et al. 2016; Mukundamago 2016; Acosta et al. 2018; Nsikani et al. 2018; du Plessis 2021). According to Holmes et al. (2020a), passive restoration results can be positive in low degradation areas, while active restoration or a combination of passive and active restoration is required in areas that are highly degraded.

Active restoration includes management of degradation drivers and to actively reintroduce native species in degraded lands to restore depleted plant communities (Cowell 2013; Atkinson & Bonser 2020; Holmes et al. 2022). However, active restoration is often labour-intensive, which makes it to be ineffective in costs and time, especially when applied at a large-scale (Bechara et al. 2021; Lehman et al. 2022; Retief et al. 2024). Alternatively, applied nucleation as a form of active restoration has been an effective tool in some types of vegetation, particularly in forest vegetation types and riparian zones (Corbin & Holl 2012; Holl et al. 2020; Rojas-Botero 2020; Bechara et al. 2021). This method better mimics the variability of natural ecosystems and uses less resources than planting the entire area (Holl et al. 2020). Applied nucleation is an approach that includes planting or sowing seeds in clusters to create nuclei which eventually expands as plants disperse over time (Corbin & Holl 2012; Holl et al. 2020; Lehman et al. 2022). Nevertheless, the outcomes of these methods are usually varied depending on the type and conditions of the ecosystems (Holmes et al. 2022). Though, the concept of applied nucleation might be the better alternative for seed limited, threatened vegetation types.

To restore ecosystems and their characteristics, whether taxonomic or functional, different approaches have emerged, each grounded in distinct principles (Mutillod et al. 2024). Aronson et al. (2020) proposed an integrated restoration plan ahead of the 'UN Decade on Ecosystem Restoration,' outlining various interlinked strategies for successful ecological restoration, namely: applying traditional ecological knowledge; integrating with movements closely aligned with restoration goals; utilizing advanced soil science technology; improving on-site training and human capacity development related to restoration; and evaluating the relationship between ecosystem restoration and social well-being. These approaches are holistic, interdisciplinary, and inclusive and nicely summarize current restoration approaches to maximize the restoration of degraded ecosystems globally but also in biodiversity hotspots like the Cape Floristic Region.

1.1.2 Restoration practices in the Cape Floristic Region

Southern Africa harbours an exceptionally rich flora characterized by high levels of endemism, with approximately 80% of the estimated 23,400 plant species being endemic to the region (Cowling et al. 1998). This remarkable biodiversity is primarily attributed to the south-western region of South Africa, identified as the Greater Cape Floristic Region (GCFR), which represents one of the world's five Mediterranean-type ecosystems (Rundel et al. 2016). The GCFR is globally recognized for its exceptional diversity of flowering plants and a high proportion of endemic species, despite its relatively small geographic size compared to other biodiversity hotspots (Myers et al. 2000). Biodiversity hotspots are defined as regions with significant levels of endemism and a high concentration of ecosystems under threat (Topp &

Loos 2019). The GCFR encompasses two major biodiversity hotspots in southern Africa: The Cape Floristic Region (CFR) and the Succulent Karoo Region (Born et al. 2007). The CFR is dominated by fynbos and renosterveld vegetation types, while the Succulent Karoo features various karoo vegetation types, including those of Namaqualand and Richtersveld (Rutherford et al. 2006). Notably, the CFR is unique and threatened by multiple factors (Topp & Loos 2018), including invasive alien species and habitat degradation, resulting in habitat loss (Holmes & Richardson 2002; Krupek et al. 2016; Mukundamago 2016; Nsikani et al. 2020; Orrock et al. 2023). These factors lead to species declines and extinction. With all these factors, van Wilgen et al. (2016) and Krupek et al. (2016) emphasized that current resources are inadequate to achieve the 30% national biodiversity conservation target for some CFR vegetation types. Therefore, while conservation should remain a priority, restoring degraded areas in the CFR is essential to help achieve the overall conservation targets.

Restoration practices that are effective in other biomes may yield different outcomes in Mediterranean systems, as ecosystems and their species respond uniquely to restoration techniques (Hobbs & Crammer 2007; Holmes et al. 2022). Seed-based restoration has been optimized globally by improving seed quality, applying enhancement techniques like coating and priming, and selecting locally adapted species (Merritt & Dixon, 2011; Pedrini et al., 2020). Direct seeding and transplanting seedlings are the primary methods used for actively restoring CFR vegetation (Iponga et al. 2005; Ruwanza et al. 2013). Amongst these, seed sowing is often preferred due to its potential to reintroduce a greater number of species at a lower cost than planting (Holmes et al. 2020a; Araujo & Perez 2022). However, restoration through seeding depends on understanding germination requirements, existing soil seed banks, and appropriate artificial pre-sowing treatments (Araujo & Perez 2022; Rashid et al. 2023). Furthermore, the success of seed sowing can be limited by several factors, including inadequate seed sources, competition with invasive alien plants, seed predation, rainfall variabilities, drought and temperatures, all of which must be thoroughly understood and managed for successful restoration (Orrock et al. 2023; Ruwanza 2017). Orrock et al. (2023) emphasized that these limitations can act individually or synergistically, complicating restoration efforts. Therefore, for restoration to be successful, effective recruitment and propagation strategies must be identified and implemented (Holmes et al. 2020a, 2020b). Furthermore, selecting appropriate species that can enhance plant diversity and contribute to long-term restoration success is critical. Evaluating soil conditions, germination requirements, and restoration methods is equally important for achieving the desired restoration outcomes (Cowell 2013). However, for renosterveld these remain largely unknown, despite the importance of restoration in renosterveld.

1.1.3 Renosterveld vegetation

Renosterveld historically covered approximately 25% of the CFR (O'Farrell et al. 2009). It is one of the most transformed vegetation types, remaining with less than 10% of its historical vegetation in the CFR (Topp and Loos 2019; Burghardt et al. 2021). Renosterveld is characterized by the dominance of *Dicerothamnus rhinocerotis*, previously called *Elytropappus rhinocerotis* (Mucina & Rutherford 2006), forming a fire-prone, evergreen shrubland composed of low-growing shrubs, grasses, herbaceous perennials – primarily from the Asteraceae family – and geophytes (Rutherford et al. 2006). While renosterveld is recognized as fire-prone, its dependence on fire for ecological rejuvenation remains uncertain (Cousins, Witkowski & Esler 2018). Kraaij and van Wilgen (2014) argued that renosterveld is less reliant on fire than fynbos vegetation types. Geologically, it is characterized by fertile, fine-grained soils (Rutherford et al. 2006), which is why lowland renosterveld is often considered ideal for agricultural activities (Burghardt et al. 2021). Consequently, most remaining renosterveld fragments are confined to steep, agriculturally unsuitable slopes (Curtis et al. 2024). The renosterveld is categorised into four main vegetation types – Shale Renosterveld, Granite and Dolerite Renosterveld, Alluvium Renosterveld and Silcrete and Limestone Renosterveld (Rutherford et al. 2006). Within these vegetation types, each contains various sub-vegetation types, with most of them being threatened (Rutherford et al. 2006) and in need of ecological restoration (Holmes et al. 2020a).

Renosterveld continues to face significant threats, which extends the challenges to achieve conservation targets (Topp & Loos 2019). Having less than 10% of its historical vegetation remaining, only 0.6% of renosterveld is formally protected (Cowling et al. 1999). Many renosterveld remnants remain privately owned and poorly protected, with an estimated annual loss of 1% of the remaining habitat (CCT 2018; Topp & Loos 2018, 2019; Burghardt et al. 2021). Between the years 2016 and 2020, approximately 478 hectares of renosterveld vegetation were lost (Moncrieff 2021), underscoring the urgent need for sustainable conservation across both public and private lands. While preventing further loss is essential, restoration of degraded renosterveld is equally critical (Krupek et al. 2016; Ruwanza 2017).

1.1.4 Restoring Swartland Shale Renosterveld

Amongst the 29 recognized renosterveld sub-vegetation types, four occur in the Swartland region: Swartland Shale Renosterveld (SSR), Swartland Granite Renosterveld, Swartland Silcrete Renosterveld, and Swartland Alluvium Renosterveld (Rutherford et al. 2006; Cousins 2017). All Swartland renosterveld types are classified as threatened, with three – SSR, Granite Renosterveld, and Silcrete Renosterveld – categorized as critically endangered (CR), and Swartland Alluvium Renosterveld categorised as vulnerable (VU) (Witt et al. 2019). The SSR, once widespread in the Cape Town area, has undergone severe degradation (Cape Town's Unique Biodiversity Endemic Ecosystems 2011). Swartland Shale Renosterveld has 214 threatened species, with 25 of these species restricted to the Swartland Renosterveld (Cousins 2017). It extends from the Swartland in the north to the Boland and Somerset West in the east (Mucina & Rutherford, 2006). Historically, SSR was converted to wheat production, leading to extensive loss of natural vegetation and invasion by alien species such as *Acacia saligna*, *A. cyclops, Eucalyptus sp.* and *Pinus radiatus* (Cousins 2017; City of Cape Town 2018). Currently, only 7.4% of its historical vegetation remains, mostly as small fragments in a transformed landscape (City of Cape Town 2022). The remnants of SSR continue to degrade and often contain invasive plants (Cousins 2017; Burghardt et al. 2021). While efforts to combat degradation drivers and restore renosterveld ecosystems have been initiated, soil-stored seed banks have declined and are insufficient for successful passive SSR restoration (Mukundamago 2016). Additionally, the scarcity of seeds for restoration poses a challenge to restore SSR and other degraded renosterveld vegetation types (Cowell 2007; Ruwanza 2017). Therefore, an active restoration strategy that can be effective using as few seeds as possible is required, since collecting seed is expensive, labour-intensive and time-consuming (Gerrits et al. 2023).

Knowledge about the minimum seed quantities required to restore degraded renosterveld vegetation while achieving desirable plant diversity and vegetation cover outcomes, remains limited. Sowing amount of seeds without systematic follow-up does not constitute an effective restoration strategy; continuous monitoring is essential to evaluate restoration success relative to input costs and to enable adaptive management that improves outcomes over time. Also, there is a need for research focusing on providing restoration approaches at species-specific level since plant diversity in restored SSR areas is typically low. This study investigates active restoration over time in the SSR to determine optimal seed sowing densities and aid restoration of species that are difficult to restore.

1.2 Statement of the research problem

Seed availability is often limiting when conducting restoration in the Cape Floristic Region (CFR). Collecting seeds is expensive and time-consuming; therefore, minimizing the amounts of seeds used while maximizing restoration success is critical in active restoration. Seed availability is even more limiting in renosterveld since it is critically endangered vegetation type. Current seed sowing guidelines are for fynbos vegetation (Holmes et al. 2022). Optimal seed sowing densities to restore renosterveld and reach the desired plant diversity and vegetation cover have not been evaluated. Additionally, some species are particularly challenging to restore, as many do not easily establish from seed sowing. This is most likely because of factors such as poor viability, specific germination requirements, incorrect pre-treatment methods, predation, rainfall variability, and other factors which may inhibit germination or plant emergence (Orrock et al. 2023). This complicates restoration to the full complement of plant species. Determining optimal seed sowing densities to restore SSR will help to reach the desired state of plant diversity and vegetation cover with minimum seed input, potentially alleviating seed harvesting pressure on surrounding vegetation. Also, investigating limiting factors that contribute to restoration challenges on some species will help to develop an effective restoration approach for these SSR species, which will enhance species diversity when restoring SSR and other renosterveld vegetation types.

1.3 Aims

This thesis firstly investigated restoration success over time in SSR following the standard sowing density and determine the optimal seed sowing densities to restore SSR. Additionally, the thesis aimed at developing a suitable reproduction and restoration approach for the species that are difficult to restore, to enhance plant diversity when restoring SSR. Specifically, the study intended to:

(1) determine the influence of standard sowing density on restoration success over time in SSR vegetation (Chapter 2),

(2) determine the minimum optimal seed sowing densities to restore SSR and reach the desired plant diversity and vegetation cover (Chapter 2) and,

(3) test the influence of seed viability, seed germination and smoke-treatment on species that are difficult to restore in SSR (Chapter 3).

1.4 Thesis outline

This thesis consists of four chapters. Chapter one covers a general introduction, background and literature review. Chapter two evaluates the restoration success over time in SSR following the standard sowing density and test different seed sowing densities on restoring SSR. This chapter mainly addresses the efficacy of minimum optimal seed sowing density that can be used in SSR and reach the desired restoration outcomes. Chapter three focuses on identifying species that are facing restoration challenges in SSR. The aim in chapter three is to develop a suitable reproduction and restoration approach for such species, as to enhance plant diversity for SSR restoration. Lastly, chapter four concludes and consolidates the outcomes and identifies future research avenues, as well as providing practical recommendations for the ongoing SSR restoration efforts. The content chapters are written as independent papers, which may result in some content duplication.

References

Acosta, A. L., de Carvalho, F. A. G., Leite, M. S., Saraiva, A. M. & Metzger, J. P. W. 2018. Gaps and limitations in the use of restoration scenarios: a review. *Restoration Ecology*, 26 (6), 1108–1119. https://doi.org/10.1111/rec.12882

Araujo, M. E. R. & Perez, D. R. 2022. From seed germination to established seedlings: a comparative evaluation in five shrub species and implications for seed-based restoration in arid lands. *Restoration Ecology*, 31 (6), 1–12. https://doi.org/10.1111/rec.13862

Aronson, J., Goodwin, N., Orlando, L., Eisenberg, C. & Cross, A. T. 2020. A world of possibilities: six restoration strategies to support the united nation's decade on ecosystem restoration. *Restoration Ecology*, 28 (4), 730–736. https://doi.org/10.1111/rec.13170

Atkinson, J. & Bonser, S. P. 2020. "Active" and "passive" ecological restoration strategies in meta-analysis. *Restoration Ecology*, 28 (5), 1032–1035. https://doi.org/10.1111/rec.13229

Bechara, F. C., Trentin, B. E., Engel, V. L., Estevan, D. A. & Ticktin, T. 2021. Performance and cost of applied nucleation *versus* high-diversity plantations for tropical forest restoration. *Forest Ecology and Management*, 491. https://doi.org/10.1016/j.foreco.2021.119088

Born, J., Linder, H. P. & Desmet, P. 2007. The greater cape floristic region. *Journal of Biogeography*, 34, 147–162. https://doi.org/10.1111/j.1365-2699.2006.01595.x

Brancalion, P. H. S., Schweizer, D., Gaudare, U., Mangueira, J. R., Lamonato, F., Farah, F. T., Nave, A. G. & Rodrigues, R. R. 2016. Balancing economic costs and ecological outcomes of passive and active restoration in agricultural landscapes: the case of Brazil. *Biotropica*, 48 (6), 856–867. https://doi.org/10.1111/btp.12383

Burghardt, S., Topp, E. N., Esler, K. J. & Loos, J. 2021. Limited knowledge flow among stakeholders of critically endangered renosterveld in South Africa. *Ecology and Society*, 26 (3), 17. https://doi.org/10.5751/ES-12083-260317

Byun, C., Kettenring, K. M., Tarsa, E. E. & de Blois, S. 2023. Applying ecological principles to maximize resistance to invasion in restored plant communities. *Ecological Engineering*, 190. https://doi.org/10.1016/j.ecoleng.2023.106926

Cape Town's Unique Biodiversity Endemic Ecosystems. 2011. *Biodiversity fact sheet 01: Peninsula Shale Renosterveld*. Retrieved from: cape town's unique biodiversity

City of Cape Town. 2018. *Biodiversity management branch restoration subsidiary plan: Tygerberg Nature Reserve*. Tygerberg Restoration Plan (Draft), Tygerberg Nature Reserve, Cape Town, South Africa.

City of Cape Town. 2019. *Draft Tygerberg district baseline and analysis report: state of the population and environment*. City of Cape Town, South Africa. https://vdocuments.mx/draft-tygerberg-district-baseline-and-analysis-report-draft-tygerberg-district-baseline.html?page=1.

City of Cape Town. 2022. *Tygerberg baseline and analysis report: integrated district spatial development framework and environmental management framework*. City of Cape Town, South Africa. TYGERBERG DISTRICT SDF REVIEW

Corbin, J. D. & Holl, K. D. 2012. Applied nucleation as a forest restoration strategy. *Forest Ecology and Management*, 265, 37–46. https://doi.org/10.1016/j.foreco.2011.10.013

Cousins, S. R. 2017. Renosterveld remnants of the Swartland: a rainbow of colour and rarity veiled in black. *Veld & Flora*: December 2017.

Cousins, S. R., Witkowski, T. F. & Esler, K. J. 2018. Influence of fire on critically endangered swartland shale renosterveld in the cape floristic region. *Applied Vegetation Science*, 21 (1), 144–155. https://doi.org/10.1111/avsc.12348

Cowell, C. R. 2007. Protocols for fynbos restoration. *Building a sustainable future: the role of botanic gardens.* Proceedings of the 3rd Global Botanic Gardens Congress, Wuhan, China. Retrieved from: https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Protocols+for+Fynbos+restoration&btnG=#d=gs_qabs&u=%23p%3DfKh4el7tj48J

Cowell, C. R. 2013. Investigating the most favourable seed establishment methods for restoring sand plain fynbos on old fields (master's thesis). Cape Peninsula University of Technology, Cape Town, South Africa, 1–138. Retrieved from: 198000472_Cowell_CR_2013.pdf

Cowling, R. M., Pressey, R. L., Lombard, A. T., Desmet, P. G. & Ellis, A. G. 1999. From representation to persistence: requirements for a sustainable system of conservation areas in the species-rich Mediterranean-climate desert of southern Africa. *Diversity and Distributions*, 5 (1-2), 51–71. https://doi.org/10.1046/j.1472-4642.1999.00038.x

Cowling, R. M., Rundel, P. W., Desmet, P. G. & Esler, K. J. 1998. Extraordinary high regional-scale plant diversity in southern african arid lands: subcontinental and global comparisons. *Diversity and Distributions*, 4 (1), 27–36.

Curtis, O. E., Bond, W. J. & Chimphango, S. 2024. Diversity and fire responses in renosterveld, the forgotten relation of fynbos, in southernmost Africa. *Journal of Arid Environments*, 225. https://doi.org/10.1016/j.jaridenv.2024.105261

Gerrits, G. M., Waenink, R., Aradottir, A. L., Buisson, E., Dutoit, T., Ferreira, M. C.… & Wubs, E. R. J. 2023. Synthesis on the effectiveness of soil translocation for plant community restoration. *Journal of Applied Ecology*, 60, 714–724. https://doi.org/10.1111/1365-2664.14364

Hall, S. A. 2018. *Determining restoration potential of alien-invaded Cape Flats Sand Fynbos; a comparison of different alien clearing treatments* (PhD Thesis). University of Stellenbosch, Stellenbosch, South Africa. Available at: https://scholar.sun.ac.za/server/api/core/bitstreams/38a8dbef-c34a-408d-b230-37dc6c358315/content

Hall, S. A., Holmes, P. M., Gaertner, M. & Esler, K. J. 2021. Active seed sowing can overcome constraints to passive restoration of a critically endangered vegetation type. *South African Journal of Botany*, 138, 249–261. https://doi.org/10.1016/j.sajb.2020.12.020

Hobbs, R. J. & Crammer, V. A. 2007. Old field dynamics: Regional and local differences and lessons for ecology and restoration. In Cramer, V. & Hobbs, R. (Eds.) *Old Fields; Dynamics and Restoration of Abandoned Farmland.* Washington, DC, Island Press, 309–318.

Holl, K. D., Luong, J. C. & Brancalion, P. H. S. 2022. Overcoming biotic homogenization in ecological restoration. *Trends in Ecology & Evolution*, 37 (9), 777–788. https://doi.org/10.1016/j.tree.2022.05.002

Holl, K. D., Reid, J. L., Cole, R. J., Oviedo-Brenes, F., Rosales, J. A. & Zahawi, R. A. 2020. Applied nucleation facilitates tropical forest recovery: Lessons learned from a 15-year study. *Journal of Applied Ecology*, 57, 2316–2328. https://doi.org/10.1111/1365-2664.13684

Holmes, P. M. 2002. Renosterveld restoration on cultivated lands at eerste river, western cape: preliminary findings of a pilot study. *Unpublished report, Lafarge Quarries*.

Holmes, P. M. & Richardson, D. M. 2002. Protocols for restoration based on recruitment dynamics, community structure, and ecosystem function: perspectives from South African Fynbos. *Restoration Ecology,* 7 (3), 215–230. https://doi.org/10.1046/j.1526-100X.1999.72015.x.

Holmes, P. M., Esler, K. J., Gaertner, M., Geerts, S., Hall, S. A., Nsikani, M. M.... & Ruwanza, S. 2020a. Biological Invasions and Ecological Restoration in South Africa. In: van Wilgen, B., Measey, J., Richardson, D., Wilson, J., Zengeya, T. (eds) Biological Invasions in South Africa. *Invading Nature - Springer Series in Invasion Ecology*, 14, 665–700. Springer, Cham. https://doi.org/10.1007/978-3-030-32394-3_23

Holmes, P. M., Esler, K. J., Gaertner, M., van Wilgen, B. W. & Richardson, D. M. 2020b. Ecological restoration of ecosystems degraded by invasive alien plants in South African Fynbos: Is spontaneous succession a viable strategy? *Transactions of the Royal Society of South Africa*, 75 (2), 111–139. https://doi.org/10.1080/0035919X.2020.1781291

Holmes, P. M., Esler, K. J., Geerts, S., Ngwenya, D. K., Rebelo, A. G., Dorse, C.... & Nsikani, M. M. 2022. *Guidelines for Restoring Lowland Sand Fynbos Ecosystems*. Self-published. ISBN: 978-0-620-98765-3.

Horn, A., Krug, C. B., Newton, I. P. & Esler, K. J. 2011. Specific edge effects in highly endangered Swartland Shale Renosterveld in the Cape region. *Mediterranean Ecology*, 37 (2), 63–74.

Iponga, D. M., Krug, C. B. & Milton, S. J. 2005. Competition and herbivory influence growth and survival of shrubs on old fields: implications for restoration of Renosterveld shrubland. *Journal of Vegetation Science*, 16, 685–692.

Kraaij, T. & Van Wilgen, B. W. 2014. Drivers, ecology, and management of fire in fynbos. In N. Allsopp, J. F. Colville, & G. A. Verboom (Eds.), *Fynbos: Ecology, Evolution, and Conservation of a Megadiverse Region*, 47–72. Oxford, UK: Oxford University Press. http://dx.doi.org/10.1093/acprof:oso/9780199679584.003.0003

Krupek, A., Gaertner, M., Holmes, P. M & Esler, K. J. 2016. Assessment of post-burn removal methods for *Acacia Saligna* in Cape Flats Sand Fynbos, with Consideration of Indigenous Plant Recovery. *South African Journal of Botany*, 105, 211–17.

Lehman, T. W. K., Esler, K. J. & Holmes, P. M. 2022. *Exploring the concept of applied nucleation as a restoration tool in a previously invaded Mediterranean climate vegetation type* (master's thesis). Stellenbosch University, Stellenbosch, South Africa. https://scholar.sun.ac.za/server/api/core/bitstreams/7d5d784d-8dab-42ab-8300-be9484b1be3a/content

Merritt D. J. & Dixon, K. W. 2011. Restoration seed banks – a matter of scale. *Science*, 332 (6028), 424–425. https://doi.org/10.1126/science.1203083

Moncrieff, G. R. 2021. Locating and dating land cover change events in the renosterveld, a critically endangered shrubland ecosystem. *Remote Sensing*, 13, 834. https://dx.doi.org/10.3390/rs13050834

Monstert, E., Gaertner, M., Holmes, P. M., Rebelo, A. G. & Richardson, D. M. 2017. Impacts of invasive alien trees on threatened lowland vegetation types in the cape floristic region, South Africa. *South African Journal of Botany*, 108, 209–222. http://dx.doi.org/10.1016/j.sajb.2016.10.014

Mucina, L. & Rutherford, M. C. (eds). 2006. The vegetation of South Africa, Lesotho and Swaziland. *Strelitzia,* 19. South African National Biodiversity Institute, Pretoria, South Africa.

Mukundamago, M. 2016. *Restoration of cape flats sand fynbos: the significance of pre-germination treatments and moisture regime* (master's thesis). University of Stellenbosch, Stellenbosch, South Africa.

Mutillod, C., Buisson, E., Mahy, G., Jaunatre, R., Bullock, J. M., Tatin, L. & Dutoit, T. 2024. Ecological restoration and rewilding: two approaches with complementary goals? *Biological Reviews*, 99, 820–836. https://doi.org/10.1111/brv.13046

Myers, N., Mittermeier, R. A., Mittermeier, C. G., da Fonseca, G. A. B. & Kent, J. 2000. Biodiversity hotspots for conservation priorities. *Nature*, 403 (6772), 853–858. https://doi.org/10.1038/35002501

Nsikani, M. M., Anderson, P., Bouragaoui, Z., Geerts, S., Gornish, E. S., Kairo, J. G.… & Peer, N. 2022. UN decade on ecosystem restoration: key considerations for Africa. *Restoration Ecology*, 31 (3). https://doi.org/10.1111/rec.13699

Nsikani, M. M., Geerts, S., Ruwanza, S. & Richardson, D. M. 2020. Secondary invasion and weedy native species dominance after clearing invasive alien plants in South Africa: Status quo and prognosis. *South African Journal of Botany*, 132, 338–345. https://doi.org/10.1016/j.sajb.2020.05.009

Nsikani, M. M., van Wilgen, B. W., Bacher, S. & Gaertner, M. 2018. Re-establishment of *Protea repens* after clearing invasive *Acacia saligna*: consequences of soil legacy effects and a native nitrophilic weedy species. *South African Journal of Botany*, 116, 103–109. https://doi.org/10.1016/j.sajb.2018.02.396

O'Farrell, P.J., Donaldson, J. S. & Hoffman, M. T. 2009. Local benefits of retaining natural vegetation for soil retention and hydrological services. *South African Journal of Botany*, 75 (3), 573–583. https://doi.org/10.1016/j.sajb.2009.06.008

Orrock, J. L., Brudvig, L. A., Damschen, E. I., Mattingly, W. B., Cruz, J., Veldman, J. W.… & Larsen-Grey, A. L. 2023. Long-term, large-scale experiment reveals the effects of seed limitation, climate, and anthropogenic disturbance on restoration of plant communities in a biodiversity hotspot. *Proceedings of the National Academy of Sciences*, 120 (7). https://doi.org/10.1073/pnas.2201943119

Ostoja, S. M., Schupp, E. W., Durham, S. & Klinger, R. 2013. Seed harvesting is influenced by associational effects in mixed seed neighbourhoods, not just by seed density. *Functional Ecology*, 27, 775–785. https://doi.org/10.1111/1365-2435.12091

Pedrini, S., Balestrazzi, A., Madsen, M. D., Bhalsing, K., Hardegree, S. P., Dixon, K. W. & Kildisheva, O. A. 2020. Seed enhancement: getting seeds restoration-ready. *Restoration Ecology*, 28 (3), 266–275. https://doi.org/10.1111/rec.13184

Piaia, B. B., Rovedder, A., Giacomini, I. F., Felker, R. M., Stefanello, M. M. Hummel, R. & Schenato, R. B. 2022. Direct seeding for forest restoration in southern Brazil: influence of soil conditions and subtropical climate. *Revista Acta Ambiental Catarinense*, 20 (1), 01–12. https://doi.org/10.24021/raac.v20i1.6142

Rashid, S., Rashid, K., Ganie, A. H., Nawchoo, I. A. & Khuroo, A. A. 2023. Seed ecology enlightens restoration of endemic species: A case study of *Actaea kashmiriana* from the Himalaya. *Ecological Engineering*, 187. https://doi.org/10.1016/j.ecoleng.2022.106880

Retief, L., Nsikani, M. M. & Geerts, S. 2024. Using fire, planting, and topsoil translocation for restoration in South African Fynbos: assessing the success and cost-effectiveness of various restoration treatments. *Restoration Ecology*, 32 (4). https://doi.org/10.1111/rec.14102

Rojas-Boteroa, S., Solorza-Bejaranob, J., Kollmanna, J. & Teixeira, L. H. 2020. Nucleation increases understory species and functional diversity in early tropical forest restoration. *Ecological Engineering*, 158. https://doi.org/10.1016/j.ecoleng.2020.106031

Rundel, P. W., Arroyo, M. T. K., Cowling, R. M., Keeley, J. E., Lamont, B. B. & Vargas, P. 2016. Mediterranean biomes: evolution of their vegetation, floras, and climate. *Annual Review of Ecology, Evolution, and Systematics*, 47, 383–407. https://doi.org/10.1146/annurev-ecolsys-121415-032330

Rutherford, M. C., Mucina, L. & Powrie, L. W. 2006 (Reprint 2011). Biomes and bioregions of southern Africa. In: L. Mucina, L. and Rutherford, M.C. (eds), *The vegetation of South Africa, Lesotho and Swaziland. Strelitzia,* 19. South African National Biodiversity Institute, Pretoria.

Ruwanza, S. 2017. Towards an integrated ecological restoration approach for abandoned agricultural fields in renosterveld, South Africa. *South African Journal of Science*, 113, 1–4. https://doi.org/http://doi.org/10.17159/sajs.2017/a0228.

Ruwanza, S., Gaertner, M., Esler, K. J & Richardson, D. M. 2013. The Effectiveness of Active and Passive Restoration on Recovery of Indigenous Vegetation in Riparian Zones in the Western Cape, South Africa: A Preliminary Assessment. *South African Journal of Botany*, 88, 132–41. http://dx.doi.org/10.1016/j.sajb.2013.06.022.

Society for Ecological Restoration International Science & Policy Working Group. 2004. The SER international primer on ecological restoration. Society for Ecological Restoration International, Tucson, Arizona. ser_primer.pdf

Topp, E. N. & Loos, J. 2018. Local and landscape level variables influence butterfly diversity in critically endangered South African renosterveld. *Journal of Insect Conservation*, 23, 225–237. https://doi.org/10.1007/s10841-018-0104-6

Topp, E. N. & Loos, J. 2019. Fragmented landscape, fragmented knowledge: a synthesis of renosterveld ecology and conservation. *Environmental Conservation*, 46, 171–179. https://doi.org/10.1017/S0376892918000498

United Nations Environment Assembly (UNEA). 2019. Resolution 73/284: United Nations Decade on Ecosystem Restoration (2021–2030). https://undocs.org/A/RES/73/284 (accessed 31 May 2021)

Witt, C. V., Holmes, P. M. & Carrick, P. 2019. *Fynbos life: Swartland shale renosterveld.* Swartland Shale Renosterveld (fynboslife.com). Accessed on 13/05/2022

van Wilgen, B. W., Fill, J. M., Baard, J., Cheney C., Forsyth, A. T. & Kraaij, T. 2016. Historical costs and projected future scenarios for the management of invasive alien plants in protected areas in the Cape Floristic Region. *Biological Conservation*, 200, 168–177. http://dx.doi.org/10.1016/j.biocon.2016.06.008

Zengeya, T. A. & Wilson, J. R. (eds). 2023. *The status of biological invasions and their management in South Africa in 2022*. South African National Biodiversity Institute, Kirstenbosch and DSI-NRF Centre of Excellence for Invasion Biology, Stellenbosch, 122. http://dx.doi.org/10.5281/zenodo.8217182

CHAPTER TWO: Determining optimal seed sowing densities for active restoration in degraded Swartland Shale Renosterveld

Abstract

Seed sowing, as part of active restoration, remains an efficient tool to restore degraded ecosystems. However, seed availability to restore degraded areas at large scale remains a limiting factor. Hence, there is an urgent need to optimize seed application during restoration while maintaining maximum ecosystem recovery. This study investigated the restoration outcome after three years using current standard seed sowing densities, and the effect of different seed sowing densities in restoring the highly threatened Swartland Shale Renosterveld (SSR) vegetation. The study was conducted at Tygerberg Nature Reserve in the fynbos biome of South Africa. Four different seed sowing densities were tested, 50%, 75%, standard (100%) and 125% with no seed sowing as a control. In total, 20 x 72 m^2 circular plots were seeded with 41 species and 5 plots acted as controls. Restoration success was quantified as species richness, vegetation cover, and species abundance. For three years of using standard seed sowing density, there was no significant change on species richness or abundance, while vegetation cover increased significantly between the first and second years. Sowing density had no significant effect on species richness, vegetation cover or species abundance, except for where natural recruitment was tested (the control), which had significantly lower recruitment. Only for *Leysera tenella* the abundance increased as sowing density increases. This study showed that by even using half the recommended seed amounts for the 14 species that recruited, similar restoration outcomes can be achieved. Therefore, fewer seeds (i.e., ≤ 65kg / ha) can be used to restore SSR ecosystems, which would also reduce the impact of seed harvesting on the SSR remnants. Additionally, the 14 of 41 species that successfully recruited highlights the need to explore reasons for low recruitment on the other species.

2.1 Introduction

It is now well established that passive restoration often does not lead to the complete recovery of highly degraded ecosystems (Cowell 2007; Brancalion et al. 2016; Mukundamago 2016; Acosta et al. 2018; du Plessis 2021). Hence, active restoration remains a restoration application with the potential to achieve desired restoration outcomes over large scales (Rashid et al. 2023). Seed application is commonly preferred for active restoration, as it has the potential to easily re-introduce more species and genetic diversity (Holmes et al. 2020). However, the input costs and, in particular, minimal seed inputs while still maximising restoration success has received limited attention (Holl et al. 2022). Practicing ecological restoration with an ideal seed sowing density ensures that resources are used optimally (Cardoso et al. 2021). Collecting seeds is often labour-intensive, time-consuming and expensive. Also, it is challenging to obtain sufficient seeds for large scale restoration in threatened vegetation types with little natural vegetation remaining. Hence, the availability of seeds to restore degraded lands at large scale remains a limiting factor in ecological restoration (Cowell 2007).

In ecological restoration, diverse mixtures of plant species can be achieved by sowing cocktails of seeds with different species at the same time. These seed cocktails are measured in different ways, while some studies use seed weights (densities) others use seed counts to make up seed mixtures. For example, Vallentine (1989) used seed counts for grasses and herbaceous perennials, which for grasses was suggested to be 200–400 seeds / m^2 and for herbaceous perennials about 100–300 seeds / m^2. While Hull (1972) and Kilcher and Heinrichs (1968) used seed weights, suggesting that for large seeds about 5–10 kg / ha should be used and for small seeds about 0.03–3 kg / ha. Using seed counts may be effective for uniform, relatively large seeds if only a few species are included. Where seed cocktails include many species, and small seeds, it is time-consuming and labour-intensive to count seeds per species (Ostoja et al. 2013). The number of seeds required per species in such a seed cocktail to restore degraded lands at large scales is therefore usually unknown (Byun et al. 2020), especially for shrubland ecosystems (Orrock et al. 2023).

Most Cape Floristic Region (CFR) ecosystems, particularly renosterveld, are highly degraded due to agricultural activities and invasive alien plants (Hall et al. 2021). To restore such ecosystems is expensive and time-consuming, since seeds are depleted. Hence, there is an urgent need to optimize seed requirements for ecological restoration while maintaining maximum ecosystem recovery. Various seed sowing densities were previously applied in restoring shrubland ecosystems (Table S1 (Annexure 1)). From all these previously applied sowing densities, only the fynbos ecosystem had a recommended sowing density that was specifically developed as part of restoration guidelines (Holmes et al. 2022). The recommended sowing density to restore fynbos vegetation is about 10 kg / ha of cleaned seeds, with species ratios based on prevailing species with good quality seeds (Holmes et al. 2022). Where dispersal structures cannot be cleaned off and influences seed weight, the sowing density is adjusted upwards accordingly. Krug (2004) developed sowing guidelines for renosterveld, but the guidelines cover sowing methods and period of sowing per species, and seed sowing densities are not specified. Sowing densities of 50 kg / ha uncleaned seeds (Holmes 2002) and 300 kg / ha uncleaned seeds (Holmes 2005) were applied in renosterveld and transitions between Succulent Karoo and Fynbos respectively. These applications factor in seeds lost during germination and establishment stages (Cowell 2013). In Tygerberg Nature Reserve, which comprises Swartland Shale Renosterveld (SSR) vegetation type, similar guidelines are followed with a standard sowing density of 950 g / 72 m^2 (which equates to 131.25 kg / ha) dried and mixed uncleaned seeds (Mamabolo & Langton pers. comm. 2022). Whether these guidelines are accurate remains largely unknown. Similarly, whether this sowing density results in the desired restoration outcomes over time is unknown. Sowing seeds every year without systematic follow-up is not an effective restoration strategy; restoration success needs to be systematically assessed relative to input costs to support adaptive management for improved long-term outcomes. High-density sowing may improve restoration (Wang 2019) but raises costs due to seed collection time and losses to seed predators. While sowing at low density will be cost-effective, but may result in unsatisfactory restoration outcomes, especially if seed mixes include seeds with low vigour (Cardoso et al. 2021 & Marcos-Filho 2015). Hence, there is a need to investigate optimal seed sowing densities for renosterveld vegetation.

This study investigated restoration success over time in SSR following the current standard sowing density and the influence of different seed sowing densities on restoring SSR. Specifically, the study intended to (1) determine restoration success over time in SSR vegetation under the standard sowing density and, (2) determine the minimum optimal seed sowing densities to restore SSR and reach the desired state of plant diversity and vegetation cover. This study used species richness, vegetation cover and abundance to measure restoration success in SSR (Ruwanza 2017; Holl et al. 2022; Holmes et al. 2022). Consequently, the study hypothesised (1) an increase in species richness, cover of native species and species abundance as the time after sowing increases and, (2) an increase in species richness, cover of native species and species abundance as sowing density increases (Burton et al. 2006; Kirmer et al. 2012).

2.2 Methods

2.2.1 Study area

This study was conducted at Tygerberg Nature Reserve (TNR), within the Fynbos biome in South Africa (-33.874835º S 18.596143º E; Figure 2.1). The TNR is located on the Tygerberg

Hills, covering an area of 388 ha (City of Cape Town 2018). The TNR vegetation (SSR) has historically been ploughed on the lower slopes, which after agricultural activities were abandoned led to invasion by alien grasses (*Cenchrus clandestinum* and *Cenchrus setaceum*) and invasive alien trees (*Acacia saligna*, *Acacia cyclops, Eucalyptus sp.* and *Pinus radiatus*) (CoCT 2018). The land was later secured for a nature reserve (TNR) in 1973 (CoCT 2010), which then included these degraded lands from which invasive trees were cleared, but the invasive grasses remained. Experiments were conducted on these degraded patches that are invaded by alien grasses (Figure 2.1).

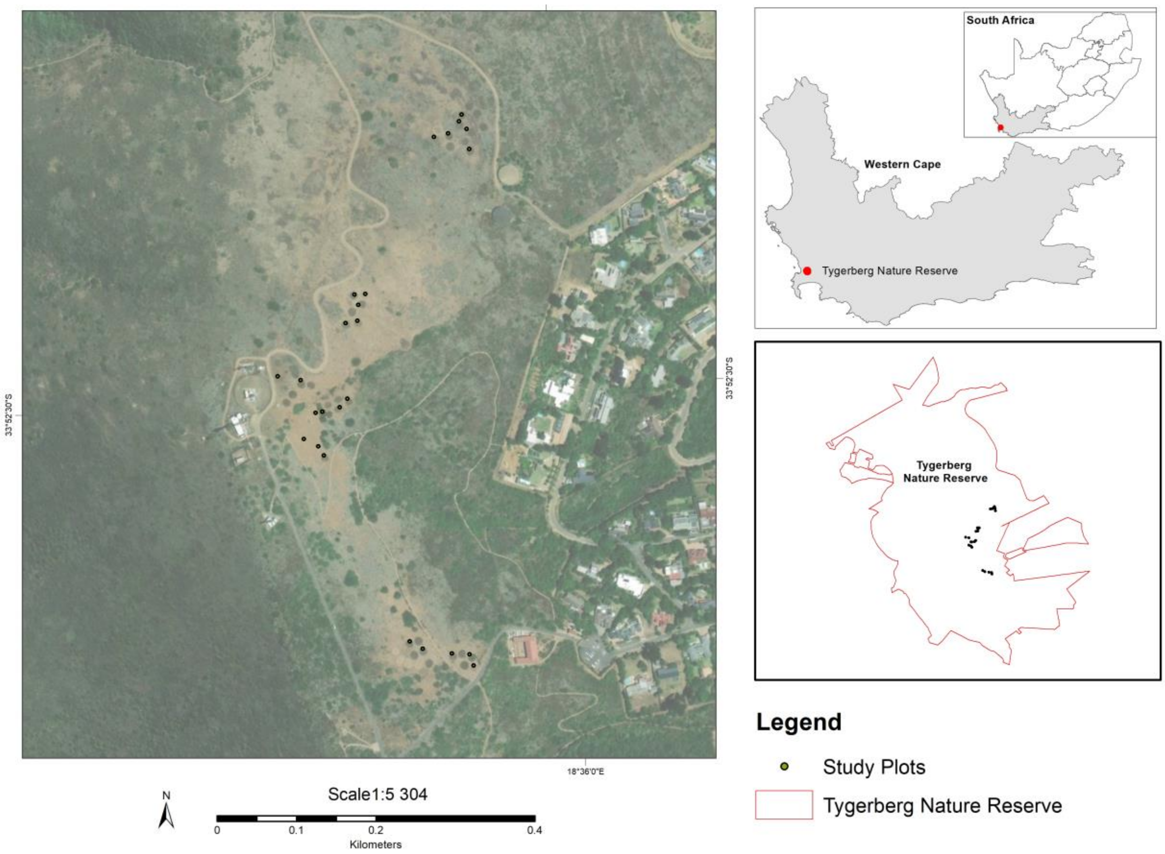

Figure 2.1: Study area for restoration experiments at Tygerberg Nature Reserve.

The TNR conserves the largest remainder of SSR vegetation (Horn et al. 2011). The SSR is dominated by varieties of low to moderately tall shrubland, as well as open shrubland dominated by *Dicerothamnus rhinocerotis* (Mucina & Rutherford 2006). The reserve contains about 460 different plant species, with three of the species being restricted to Tygerberg region, and twelve species threatened with extinction (CoCT 2019). The SSR consists of fertile fine-grained soils which are derived from the Malmesbury Group Shales (Witt, Holmes & Carrick

2019). The climate is Mediterranean, with winter rainfall between May and August, with a mean annual rainfall of about 430 mm (Mucina & Rutherford 2006; Witt et al. 2019). Average daily maximum and minimum temperatures are 29.6ºC and 6.3ºC for summer and winter, respectively (Mucina & Rutherford 2006).

2.2.2 Restoration success over time in SSR

The TNR has previously carried out active restoration to restore degraded SSR lands inside the reserve. Different restoration plots (72 m^2) sown for three consecutive years (2020 = 6 plots sown, 2021 = 6 plots sown and 2022 = 5 plots sown) following the standard sowing density (950 g / 72 m^2) were sampled in the summer of 2022 to determine restoration success over time. The species sown throughout these years are presented in Table S1, 32 species sown in the year 2020, 29 species in 2021 and 41 species in 2022. A space for time or chrono sequence approach was used with the years since sowing categorised relative to the sampling year, i.e., 2020 as year 3 post-sowing, 2021 as year 2 post-sowing, and 2022 as year 1 post-sowing. Water supply for germination and emergence was dependent on rain events that took place, since sowing was done prior to rain season in the Cape Floristic Region (CFR) (June – August). Vegetation cover was estimated by three individual recorders and the mean values used. Species abundance was determined by counting the number of individual plants per species. For species richness, plant surveys were done in winter, spring, and summer of 2022. In cases where plant identification needed verification or confirmation, iNaturalist and external experts were utilised.

2.2.3 Seed sowing densities

Circular field plots (72 m^2; n = 25) were used to test the different seed sowing densities and compare with the standard sowing density utilised in SSR. Plots were distributed randomly as to have different slopes and aspects (Figure 2.1). Each treatment and control method were represented by five (5) replicates in each slope and aspect. Plot preparations included clearing of alien grasses mechanically. Being historical agricultural land, the soil was tilled and loosened using hand tools, to enhance aeration and drainage to support germination of seeds and survival of seedlings. No organic or chemical supplements were added to the soil.

Seeds used for this experiment were all collected within TNR. Seeds were treated with smoke at Westlake Conservation Centre. Seed smoking entailed setting out seeds in flat trays per species, then placed inside an enclosed plastic tank. A mixture of dry and green fynbos plant material was ignited inside the metal drum, and the resulting smoke was pumped through a chimney into the plastic tank (adapted from Holmes et al. 2022). Seed trays were left inside the plastic tank for about three hours to allow smoke to settle over the seeds. In total, 41 different species which are native to SSR were included in the standard mixture of seeds (Table S2). Seed mixtures were then prepared with equal proportions of each species across varying sowing densities, accounting for differences in seed weights among species. As a result of limited seed availability, the seed mixture for each sowing density, seed amounts differed between species.

With weather conditions favouring sowing later than mid-autumn, due to delayed rains, sowing was done in early June 2022. Four different seed sowing densities, 50%, 75%, standard (100%), and 125% (475 g, 713 g, 950 g and 1188 g) were sown (hand broadcasted evenly) in five plots per treatment. These seed sowing densities were established in comparison to the standard and currently applied density. As a control, no seeds were sown to control for potential soil-stored seed banks and natural dispersal. A total of 16.63 kg of seeds was sown across all treatments. To ensure minimal disturbance inside plots, weeds or invasive plants were not controlled after seed sowing. The experiment was designed to be scalable and as a result, animals (most specifically rodents and granivores) were not excluded from the plots. Also, no irrigation or moisture-holding substrates were added. Water supply was dependent on the rain events taking place from June to August in the CFR.

2.2.4 Statistical analyses

Restoration success over time in SSR

To assess whether species richness, cover of native species and species abundance would increase in time since seeding, a mixed effects linear model was created. The experiment consisted of repeated measures; hence a mixed effect linear model was created using the package '*lme4*' (Bates et al. 2015). The model compared cover types: bare soil cover (clearly exposed soil), native species cover and invasive species cover (alien annuals and grasses) with years after sowing, and the interaction between cover types and years after sowing as fixed effects. A similar model was built for individual plant species. Years after sowing and replicates were included as nested random effects to account for repeated measurements. The post-hoc analysis for the two-way interaction between cover and years after sowing was calculated using package '*emmeans*' (Lenth 2024). The coefficient of determination for models was calculated using the '*MuMIn*' package (Bartoń 2023). Model validation was done through residuals, Q-Q plot, and the histogram. All the assumptions for mixed-effects model as the best fit were met.

Since data were normally distributed (Shapiro-Whilk test all $P > 0.1$), a repeated measure analysis of variance (ANOVA) was performed using the package '*rstatix'* (Kassambara 2023) to test the significance of species richness between different years after sowing. The main effects for the ANOVA were calculated using package '*car'* (Fox & Weisberg 2019).

Seed sowing densities

To test the effect of different seed sowing densities on restoration success, species richness, vegetation cover, and abundance were quantified. For this, a mixed-effect linear model was created using the '*lme4*' package (Bates et al. 2015) to compare species richness and vegetation cover across sowing densities, with cover type and sowing density as fixed effects and replicates (restoration plots) as random effects. All the assumptions for a mixed-effect linear model were validated using scatter plot of the residuals, Q-Q plots and histogram of the residuals. The mixed effect linear models were created with Kenward-Roger "F" tests and Satterthwaite's approximation for denominator degrees of freedom. The coefficient of determination value for models was calculated using the package '*MuMIn*' (Bartoń 2023). The Turkey post-hoc analysis for the two-way interaction between cover types and sowing densities was calculated using package '*emmeans*' (Lenth 2024).

A repeated measure ANOVA was performed using the package '*rstatix'* (Kassambara 2023) to test the significance of species richness between different sowing densities. The main effects were calculated using the package '*car'* (Fox & Weisberg 2019) and using a Chi-squared test for cover types and sowing densities. All statistical analyses were performed using R statistics software v 4.4.0 (R Core Team 2024) and graphics were generated using packages '*ggplot2'* (Wickham 2016) and '*ggsignif'* (Ahlmann-Eltze & Patil 2021).

2.3 Results

2.3.1 Restoration success over time in SSR

There was no significant difference in species richness between three years post sowing (repeated measure ANOVA, $F = 1.79$, $df_n = 2$, $df_d = 8$, $p = 0.228$). An average of nine species per plot was recorded over the three years. In total, 14 species (*Anthospermum spathulatum, Athanasia trifurcata, Chrysocoma coma-aurea, Dicerothamnus rhinocerotis, Eriocephalus* *africanus, Euryops linifolius, Helichrysum cymosum, Helichrysum patulum, Lessertia frutescens, Leysera tenella, Otholobium hirtum*, *Podalyria sericea, Salvia lutea-africana* and *Stoebe plumosa*) were recorded in the restoration plots. Nine species (*A. spathulatum, A. trifurcata, C. coma-aurea, E. africanus, E. linifolius, H. patulum, L. tenella, O. hirtum* and *P. sericea*) were recorded every year.

Percentage cover of native species significantly increased as invasive species cover decreased, and bare soil decreased with an increase in invasive or native species cover ($F = 64.16$, $df = 2$, $df_{res} = 28$, $p < 0.001$). Native species cover significantly increased from year one to year two but then showed no significant increase from year two to three (Figure 2.2, Figure 2.3), while invasive species cover significantly decreased from year one to year two and then showed no significant difference between years two and three (Figure 2.2). There was no significant difference in percentage bare soil between the three years after sowing (Figure 2.2).

The year after sowing showed no relationship with species abundance for native species, except for *A. trifurcata*, *C. coma-aurea* and *S. africana-lutea*, which showed an increase from year 1 to year 2 (Figure 2.4). The species *P. sericea* decreased from year 1 to year 3, while *O. hirtum* was not recorded in year 3 and *A. spathulatum* in year 2. Three species (*Dimorphotheca pluvialis*, *L. frutescens* and *S. plumosa*) were present in year 1 only.

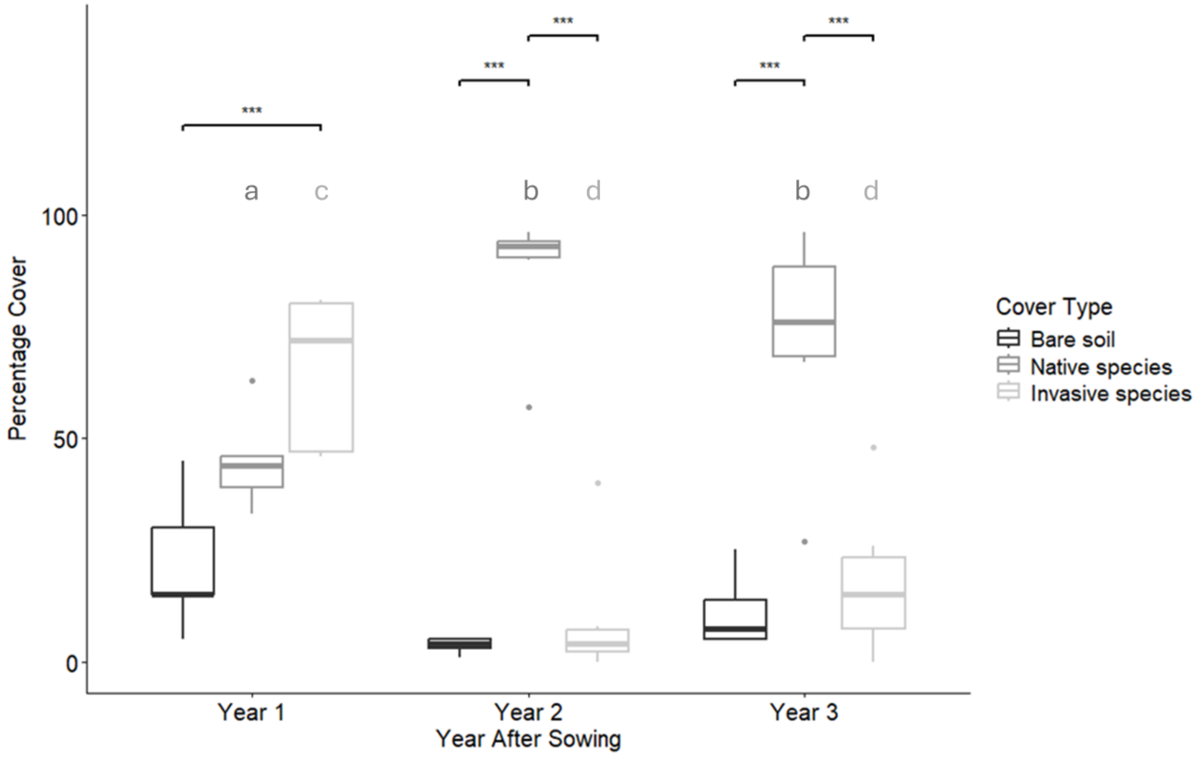

Figure 2.2: Boxplot of percentage cover by cover type for three years after sowing. The box represents the interquartile range (IQR), the middle line represents the median and the whiskers represent 1.5x IQR or range if less than these values, the points represent the median and the whiskers represent 1.5x IQR or range if less than these values, the points represent outliers. The significant difference between years in cover type is indicated by letters (same letter indicate no significant difference) and the significant difference between cover types within a year is indicated by the bars and ***. There was no difference in bare soil between years, hence no significance letters. Year 1 indicates plots sown in 2022 while year 3 indicates plots sown in 2020.

Figure 2.3: A landscape image presenting vegetation cover of restoration plots with bottom-left corner: sowed in 2022; top-left corner: sowed in 2021; top-right corner: sowed in 2020. Inserts present zoomed close-up photos.

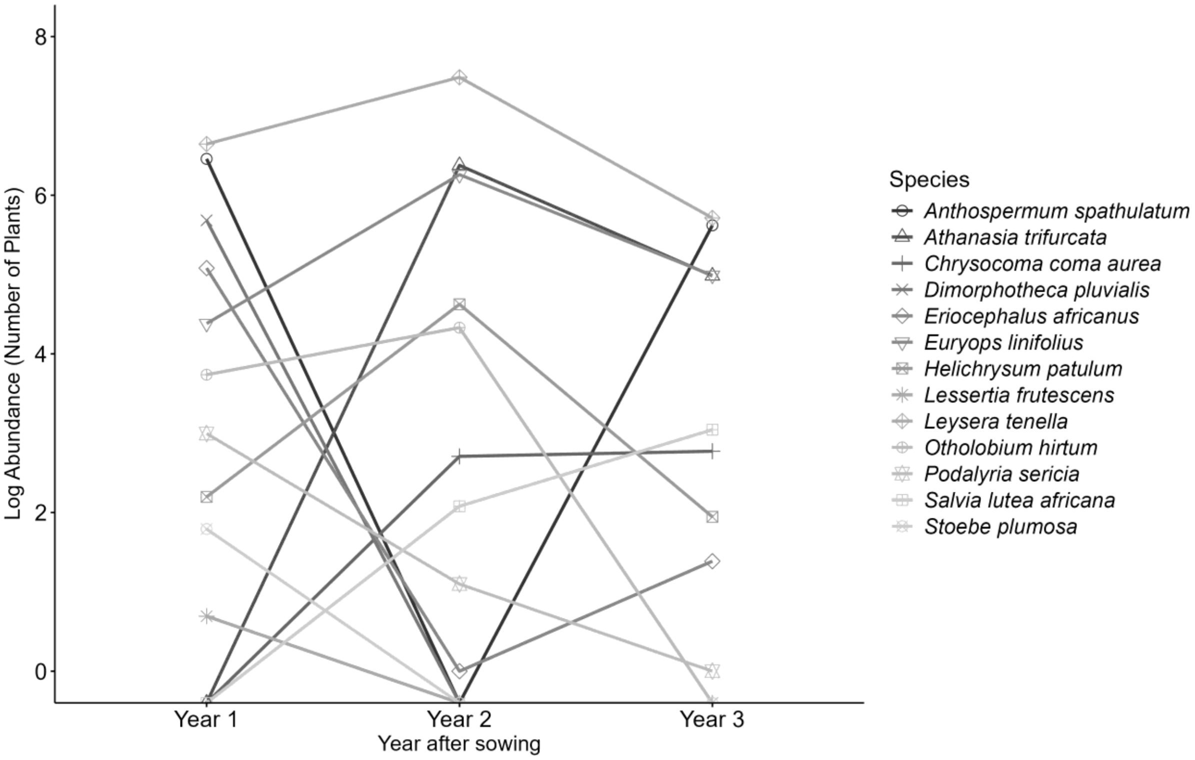

Figure 2.4: Species abundance over the three years after sowing. The lines represent the change within species with time since restoration. Species are represented by unique symbols and different shades of lines.

2.3.2 Seed sowing densities

There was no significant difference in the native species richness between different sowing densities with only the control being significantly lower than all other treatments ($F = 26.92$, $df = 4$, $df_{res} = 20$, $p < 0.001$; Figure 2.5). The average species richness, except for the control (i.e. no seeds sown), ranged between eight and nine species with a maximum of 12 species in some plots (Figure 2.5). The control had 3 species recorded across all replicates. Out of the 41 species sown, only 12 species recruited. Across all treatments, excluding the control, the same eight species (*A. spathulatum, D. pluvialis, E. africanus, E. linifolius, O. hirtum, L. tenella, P. sericea*, and *H. patulum*) occurred.

There was no significant difference in percentage cover of native species for the different seed sowing densities, except with the control, which was significantly lower than all other sowing densities (Figure 2.6). For bare soil and invasive species, there was no significant difference between the sowing densities (Figure 2.6). Pairwise comparisons of cover types across sowing densities revealed that invasive species consistently achieved significantly higher percentage cover (65–75%) than bare soil or native species (Figure 2.6).

For species abundance (number of plants), there was no relationship between sowing densities and number of plants (Figure 2.7). The lowest abundance was recorded in the control treatment. The species *A. trifurcata, H. patulum* and *O. hirtum* were the only species present in the control treatment (Figure 2.7). All species recorded in the control were also sown and present in other sowing densities. The species *C. coma-aurea, E. africanus, E. linifolius, L. frutescens*, *P. sericea* and *S. plumosa* were recorded in all sowing densities beside the control. The low recruitment could be in part be due to seed predators (Figure 2.8 a, b, c & d) and competition with alien plants (Figure 2.8 e, f).

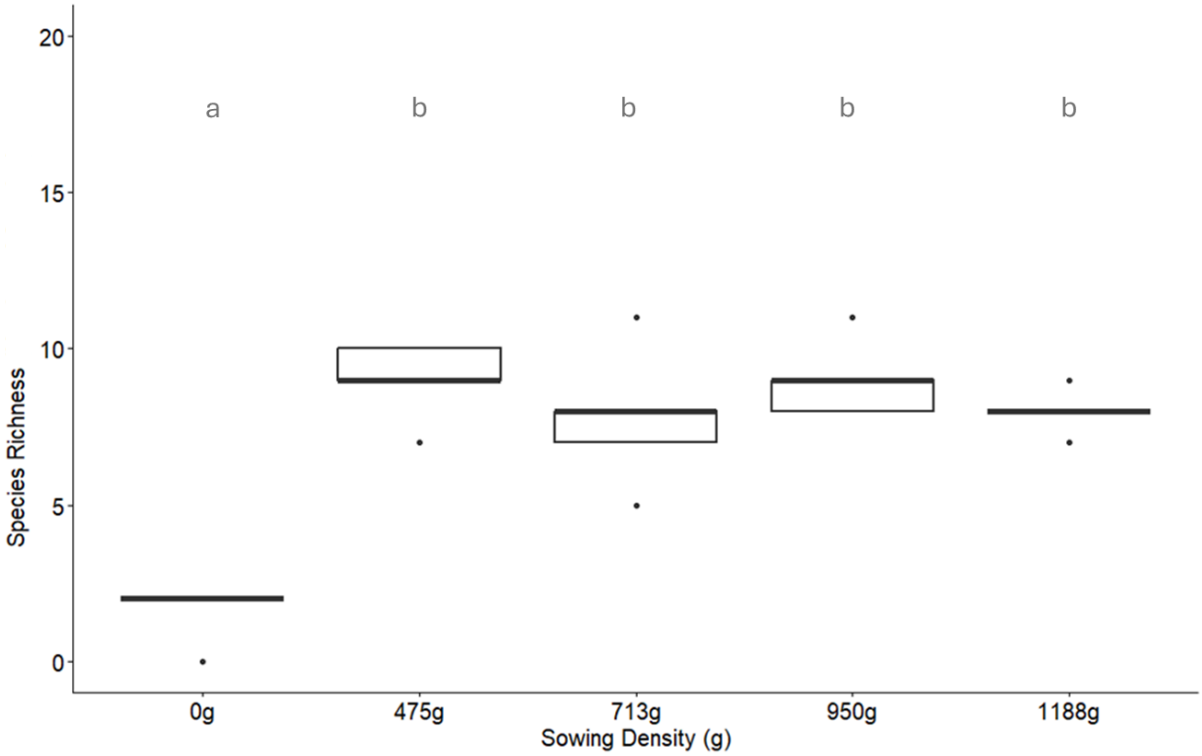

Figure 2.5: Boxplot of the species richness for native species by sowing density. The 950-gram treatment is the standard sowing density. The box represents the interquartile range (IQR), the middle line represents the median and the whiskers represent 1.5x IQR or range if less than these values, the points represent outliers. The significant difference in species richness between sowing densities is indicated by letters (same letter represent no significant difference). The density 0 g indicates no sowing and was included to control for a potential soil seed bank and seed dispersal.

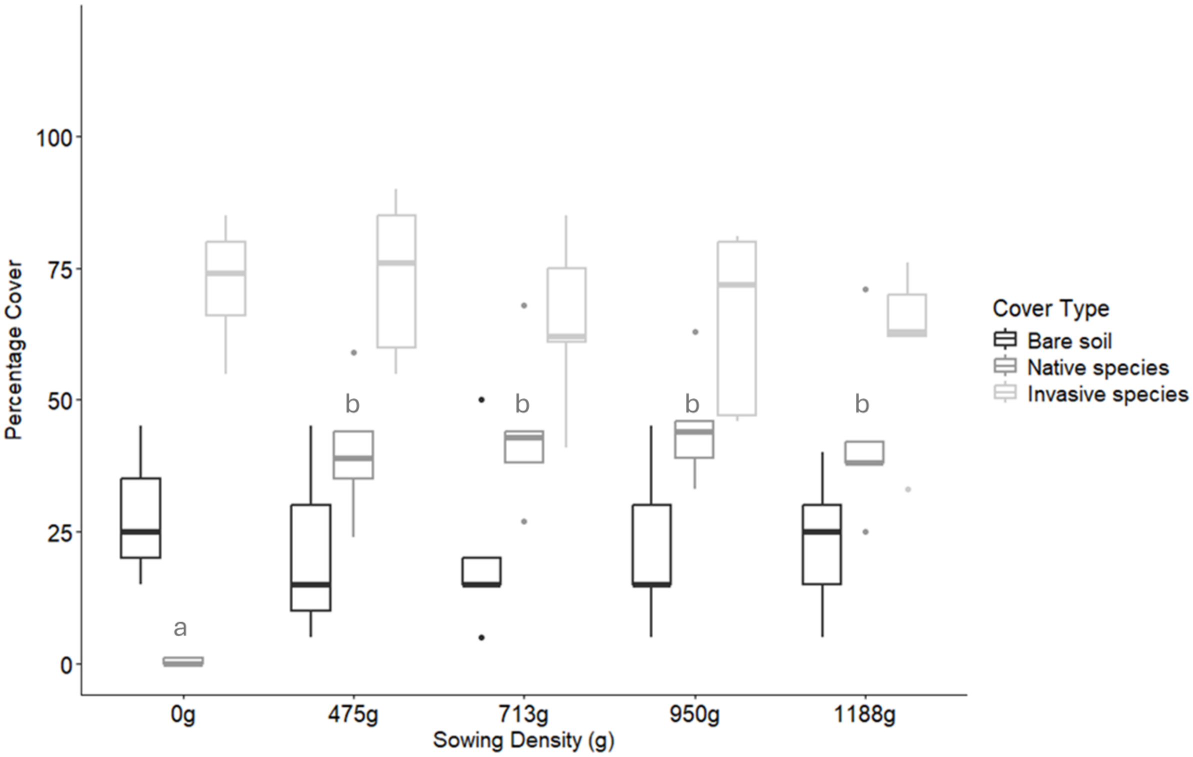

Figure 2.6: The percentage cover for different sowing density levels for native plant species, invasive plant species and bare soil. The box represents the interquartile range (IQR), the middle line represents the median and the whiskers represent 1.5x IQR or range if less than these values, the points represent outliers. A significant difference between sowing densities for native species is indicated by letters (same letter represent no significant difference). There was no difference between sowing densities for invasive species and bare soil, hence no significant letters. The density 0 g shows no sowing and was the control, while 950 g was the standard sowing density.

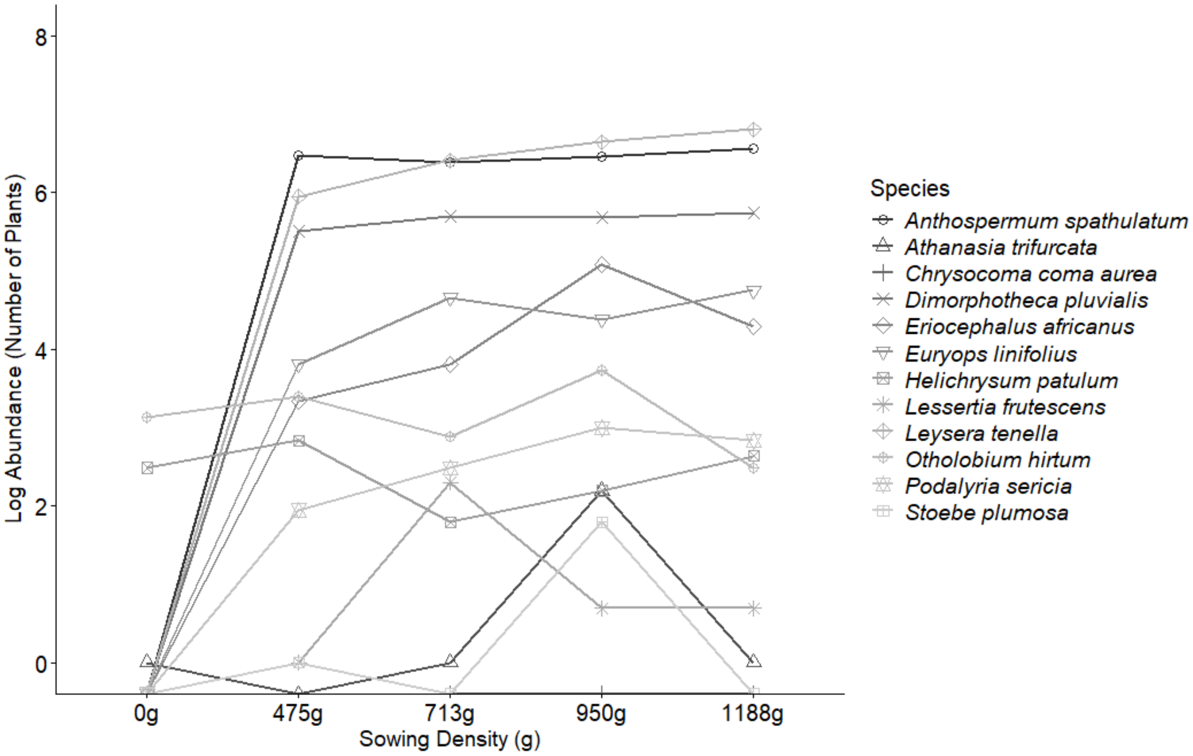

Figure 2.7: Species abundance over the five sowing densities. The lines represent a trend of change within species across the sowing densities. Species are represented by unique symbols and different shades of lines. The Y-axis (abundance) logged to account for clarity on adequate abundance between species with low number of plants and high number of plants (i.e. 20 individuals per species = adequate abundance while 600 individuals per species = over enough).

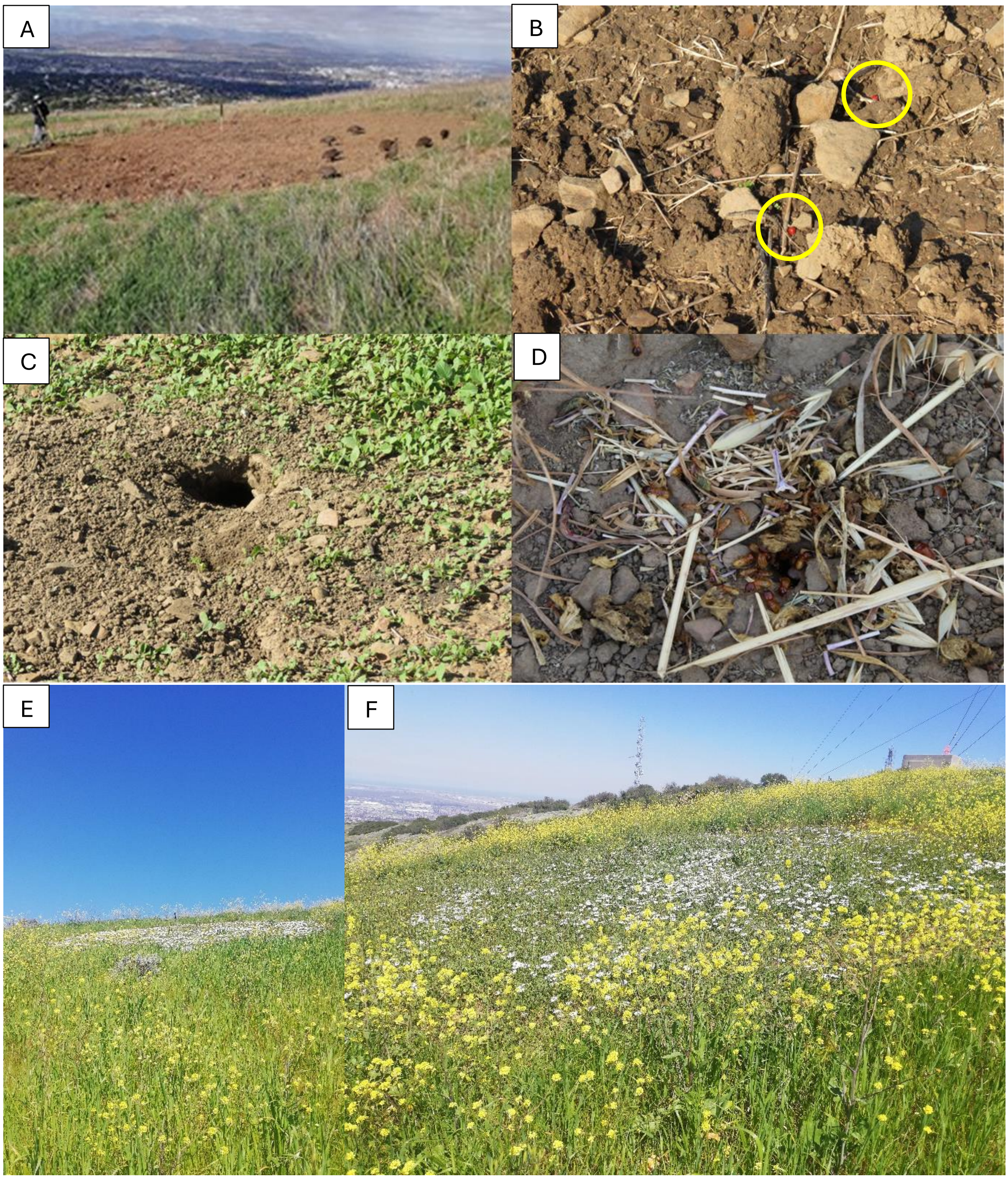

Figure 2.8: Observations captured inside the restored plots at TNR. The top four photos show the potential for seed loss by seedeaters, while the bottom two photos show the competition between native annual species and invasive annual species. A = Guinea Fowls feeding from sown plots; B = exposed seeds after heavy rainfall post seed sowing; C = rodent borrow on prepared plots; D = Termites activity in the restoration plots; E = sowed *Dimorphotheca pluvialis* plants competing against *Rapistrum rugosum* in restoration plots and F = zoomed in plot dominated by *D. pluvialis* at Tygerberg Nature Reserve (2022).

2.4 Discussion

Restoration success over time in SSR

The hypothesis on restoration success over time, following the standard seed sowing density, predicted that species richness, cover of native species and species abundance would increase from the first year after sowing. The species richness remained the same over time with an average of eight to nine species across different years. Also, with only 14 different species recorded across the three years post restoration, richness remains limited. Consequently, this study revealed similar richness results over time as the different sowing densities (9 species per plot). Across all established species, Asteraceae recorded 9 species in total, with 6 of them occurring across different years. Out of 23 families sown in total (Table S2), only four families established in this study, with 3 species from Fabaceae, 1 from Lamiaceae, 1 from Rubiaceae and the remaining species from Asteraceae. Even with Asteraceae dominating species richness in this study, the species establishment within the family varied across different years. Additionally, while some Asteraceae species had a relatively small number of seeds to establish, i.e. *Arctotis calendula, Athrixia crinata, A. capensis, Gazania krebsiana, Nidorella foetida, Osteospermum monstrosum* and *Pteronia hirsuta* (Table S2), other species i.e., *Felicia fruticosa, Helichrysum cymosum, H. dasyanthum, H. teretifolium* and *Printzia polifolia* were not recorded despite adequate seed amounts. *Helichrysum* had the most species sown from a single genus (4 species sown) but only *Helichrysum patulum* emerged but with small numbers across all years. This suggests that a limiting factor, other than seed amounts, may be affecting establishment of *Helichrysum* species compared to other Asteraceae species. Abiotic conditions, such as heat or smoke stimulation can influence species establishment during restoration (Cousins et al. 2018; Byun et al., 2023). Afolayan et al. (1997) recorded a better germination and emergence of *Helichrysum aureonitens* from non-treated seeds than smoke treated seeds, suggesting that smoke treatment may be inhibiting germination of *H. aureonitens*. In SSR, seed smoking has been a standard restoration practice, but its effect on *Helichrysum* species remains unclear. Further research is therefore needed to enhance germination and emergence of *Helichrysum* species in SSR restoration (Chapter 3).

The percentage cover of native species increased with time post restoration, while cover of invasive species decreased significantly (Figure 2.2). These results suggested that a competitive cover of native species has the potential to decrease invasion by grasses and herbs, similar to Byun et al. (2023). Comparable results were achieved by Robinson et al. (2023), with cover of invasive species declining as one of native species increased. Bare soil cover was low across all years (Figure 2.2), reflecting the inverse relationship between native species cover and invasive species cover, which consistently led to high total vegetation cover. Besides invasive grasses (*Briza maxima*, *Cenchrus clandestinum* and *Cenchrus setaceum*), invasive annuals (*Avena fatua*, *Echium plantagineum* and *Rapistrum rugosum*) dominated. These invasive grasses and annuals may have impacted the germination and emergence of some native species, reducing native species richness, particularly the first year after sowing.

The relationship between years after sowing and species abundance was predicted to be linear, with the abundance increasing as the number of years after sowing progressed. The years after sowing had no relationship with species abundance for native species, except for *Athanasia trifurcata*, *Chrysocoma coma-aurea* and *Salvia africana-lutea*, which showed an increase over time (Figure 2.4). This suggests that these species may be establishing better at least after a year cycle. The abundance of *Podalyria sericea* decreased over time (Figure 2.4), probably because of different seed amounts in different years, since year 1 comprised of larger seed amounts than year 2 and probably year 3, which was unknown (Table S2). Three species (*Dimorphotheca pluvialis*, *Lessertia frutescens* and *Stoebe plumosa*) were only present in year 1. This part of the study did not account for species-specific sowing densities across years. Since even with standard sowing density followed, the seed densities for each species were not uniform across the years and others were unknown. While the current literature favours the inclusion of specific plants in seed mixes (i.e., nurse plants and pioneer species) (Lehmann 2022), the balance in seed amounts remain unknown in renosterveld restoration. Thus, the unknown variances in seed amounts may have impacted the species abundance outcome.

Seed sowing densities

The hypotheses of this study predicted an increase in species richness, cover of native species and species abundance as the sowing density increases. The results showed no significant difference in restoration outcomes between sowing densities (475 g, 50% = 713 g, 75% = 950 g, 100% = 1188 g, 125%), except for no sow treatment, which was significantly lower than all densities. This suggests that similar restoration outcomes can be achieved using half of the recommended Swartland Shale Renosterveld (SSR) standard sowing density. The standard sowing density followed in this study has been used by Tygerberg Nature Reserve (TNR). This sowing density (131.25 kg / ha) falls between the range of previously applied sowing densities for renosterveld, 50 kg / ha (Holmes 2002) and Fynbos-Succulent Karoo transitions, 300 kg / ha (Holmes 2005). Consequently, the minimum sowing density tested in this study (65,63 kg /

ha) is advisable since an increase in sowing density has no effect on restoration outcomes. In fact, lower sowing densities should be explored.

The species richness was surprisingly low across treatments compared to the total number of species sown (Figure 2.5). Out of 41 species, only 12 species were recorded across all sowing densities. Most of these species dominated the seed mixes, with high establishment expected. Also, out of 12 species recorded, 9 were from the family Asteraceae, which also dominated the seed mixes, with 19 species included (Table S2). *Euryops linifolius* was one of the Asteraceae species that established despite low seed amounts (Table S2). *Lessertia frutescens* (Fabaceae) also established despite low seed amounts, while other species with low seed amounts failed to establish (Table S2). The species *C. coma-aurea, Otholobium hirtum, P. sericea,* and *S. plumosa* had few individuals establishing, while *Dicerothamnus rhinocerotis*, *F. fruticosa, H. cymosum*, *H. dasyanthum*, *H. teretifolium*, and *P. pilifolia* failed to establish despite having high seed amounts (Table S2). This suggests that there were other factors limiting establishment of some species, beside sowing density (Piaia et al. 2022). Orrock et al. (2023) stated that plant establishment using seed sowing as a restoration tool can differ based on soil attributes, disturbance regimes and or variation in weather conditions throughout the year. Poor or compacted soils may reduce establishment success. While soil disturbance often creates opportunities for seedling establishment, excessive disturbance may impact seedling establishment negatively. Additionally, weather patterns, particularly rainfall timing and amount, and temperature fluctuations, further drive variability in germination and early growth across years. Seed viability, appropriate sowing season, pre-treatment requirements, germination conditions, predation, early-stage competition, and prolonged dry seasons are key factors potentially influencing seedling establishment in SSR. Limited understanding of endemic species' seed ecology complicates their chances of restoration, reducing species richness (Rashid et al., 2023). Enhancing species richness in SSR restoration is essential, with investigations needed at species or family levels. Orrock (2023) found that some species showed low initial establishment but strong survival, suggesting alternative restoration methods may be more effective. Improved knowledge of seed ecology in SSR and other renosterveld vegetation types is urgently needed to optimize restoration efforts (Chapter 3).

While native species cover was significantly lower in the control, invasive species cover showed no significant difference between sowing densities and remained higher than native species cover or bare soil cover, even in the control (Figure 2.6). Probably, while the sowing density of native species affects their own cover, it does not significantly impact invasive species cover at the early recruitment stage. This is likely due to factors related to competition resilience, ecological niches, or resource availability (Nsikani et al. 2018; Ngwenya et al. 2023). The percentage cover of bare soil remained constant as well across different sowing densities. With the cover of native species dominated by perennials and shrubs seedlings, their impact on bare soil cover may be minimal at first year after sowing but changing over time (Figure 2.2).

To combat competition resilience of invasive species, it is possible that using a diversity of annual species to restore degraded SSR areas may enhance the suppression of invasive species (Hall et al. 2021; Byun et al. 2023; Robinson et al. 2023). A comparative study in fynbos by Ngwenya et al. (2023) demonstrated the cover of native annual species contributing to suppressing recruited invasive acacia seedlings, which aided in native perennials to establish themselves before native annuals die back. Similarly, the study conducted by Hall et al. (2021) showed that using native annual species for active restoration can result in having high annual forb cover and species richness. The percentage cover of invasive species, dominated by *A. fatua*, *B. maxima, E. plantagineum* and *R. rugosum*, significantly decreased from year 1 to year 2 after sowing, while cover of native species increased inversely (Figure 2.2). Out of five annual species, two (*D. pluvialis* and *Leysera tenella*) significantly contributed to percentage cover during this study, with *D. pluvialis* proving to be resilient in plots which were dominated by alien annuals and grasses (Figure 2.8).

It was hypothesized that individual species abundance would increase proportionally with higher sowing densities. The results indicated no clear relationship between native species abundance and sowing density, except for *L. tenella*, whose abundance increased proportionally with higher sowing densities. This study offered limited insights of why varying seed sowing densities have no influence on species abundance, suggesting factors which were not examined here may be influencing abundance. Factors such as predation, seed viability, aspect, slope, and other environmental variables may have influenced the observed abundance patterns in this study. Nonetheless, most species which established had at least 0.2 individuals per m^2, implying that despite the variability in response to sowing density, the establishment was sufficient to at least initiate vegetation recovery.

The influence of natural recovery, through natural seed dispersal and the soil-stored seed bank was low, only resulting in a maximum of 2% cover by native species. Ngwenya et al. (2023) reported similarly low natural recovery in Lowland Sand Fynbos, using comparable methods. Three native species were recorded growing in control plots (*A. trifurcata, H. patulum* and *O. hirtum*). Given the small, brush-like seeds of *H. patulum* and *A. trifurcata* (Asteraceae), these two species may be establishing from seeds dispersed by wind (Traveset et al. 2014; Kravtsova 2023). *Otholobium hirtum* may have established from soil-stored seed banks, as seen in relative species from the shale and granite renosterveld (Power et al. 2011). Nonetheless, both natural dispersal and soil-stored seed banks contribute very little towards SSR restoration in areas dominated by alien grasses and with an agricultural land use history.

This study did not implement animal exclusion measures following seed sowing. The study area consisted of guineafowls and spurfowls, which were observed consuming seeds inside plots immediately after sowing (Figure 2.8a). It is most likely possible that these birds consumed mostly visible seeds inside the plots, i.e. *Kiggelaria africana*, *Olea europaea* subsp. *africana* and *Searsia* species, resulting in these species failing to establish. Some larger seeds became exposed to sunlight and seed predators after the first rains (Figure 2.8b), suggesting a deeper seed burial depth might be needed to avoid exposure and enhance germination chances. In addition to predation by birds, rodents, indicated by diggings (Figure 2.8c) and termites (Figure 2.8d) were also noted inside plots. Though, it is not clear which species these seed predators may have affected. However, mitigating seed predation, such as through exclusion measures following sowing, could improve germination and establishment, particularly for species that are prone to seed predation. Collectively, seed predators have contributed to the limited restoration outcomes of this study and deserve research attention as to improve plants species richness when renosterveld is restored. The study demonstrated that sowing density alone is insufficient for successful SSR vegetation restoration, even when seed amounts are increased. Increased sowing density for some species might have improved recruitment, but this is largely unknown. Also, high-density seeding increases vulnerability to seed predation, elevating the risk of seed loss (Wang, 2019).

2.5 Conclusion

This study investigated active restoration success over time and the influence of different seed sowing densities on restoration outcomes. It was hypothesised that the species richness, vegetation cover and species abundance increase quantitatively over time and with higher sowing densities. However, there is no difference between years after sowing on species richness and no relationship on species abundance between years after sowing. The overall species richness post restoration was low. Hence, it is recommended that future research explores the germination and recruitment barriers in those species that were sown in large numbers but show no or low recruitment. The study has also proved that cover of native species in SSR changes rapidly from a year after sowing and remain consistent going forward. The study demonstrated an inverse relationship between native and invasive species cover over the three-year period. There are no significant differences in species richness and vegetation cover across different sowing densities, except for where natural recovery was tested. Natural species recruitment in this study was not effective, suggesting a minimal to no influence towards the overall restoration outcomes in the SSR vegetation. Most species studied showed no relationship between sowing density and species abundance, indicating that factors beyond sowing density influence successful restoration outcomes in SSR vegetation. Hence, other factors affecting plant germination and emergence from seeds should be investigated. The study suggests that half of the standard sowing density (i.e., ≤ 65kg / ha) may be sufficient to restore degraded SSR lands, producing comparable outcomes to the standard sowing density.

References

Acosta, A. L., de Carvalho, F. A. G., Leite, M. S., Saraiva, A. M. & Metzger, J. P. W. 2018. Gaps and limitations in the use of restoration scenarios: a review. *Restoration Ecology*, 26 (6), 1108–1119. 1108-1119. https://doi.org/10.1111/rec.12882

Afolayan, A. J., Meyer J. J. M. & Leeuwner D. V. 1997. Germination in *Helichrysum aureonitens* (Asteraceae): Effects of temperature, light, gibberellic acid, scarification and smoke extract. *South African Journal of Botany*, 63 (1), 22–24.

Ahlmann-Eltze, C. & Patil, I. 2021. ggsignif: R Package for Displaying Significance Brackets for 'ggplot2'. *PsyArxiv*. https://doi.org/10.31234/osf.io/7awm6

Bartoń, K. 2023. *MuMIn: Multi-Model Inference*. R package version 1.47.5, Available: https://CRAN.R-project.org/package=MuMIn. Accessed on 05/11/2024

Bates, D., Maechler, M., Bolker, B. & Walker, S. 2015. Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, 67 (1), 1–48. https://doi.org/10.18637/jss.v067.i01

Brancalion, P. H. S., Schweizer, D., Gaudare, U., Mangueira, J. R., Lamonato, F., Farah, F. T.... & Rodrigues, R. R. 2016. Balancing economic costs and ecological outcomes of passive and active restoration in agricultural landscapes: the case of Brazil. *Biotropica*, 48 (6), 856–867. https://doi.org/10.1111/btp.12383

Burton, C. M., Burton, P. J., Hebda, R. & Turner, N. J. 2006. Determining the optimal sowing density for a mixture of native plants used to revegetate degraded ecosystems. *Restoration Ecology*, 14 (3), 379–390.

Byun, C., Kettenring, K. M., Tarsa, E. E. & de Blois, S. 2023. Applying ecological principles to maximize resistance to invasion in restored plant communities. *Ecological Engineering*, 190. https://doi.org/10.1016/j.ecoleng.2023.106926

Byun, C., Oh, M., Lee, E. J. & Kang, H. 2020. Seed density is as important as limiting similarity, diversity effect, and propagule pressure in plant restoration to control invasion. *Ecological Engineering*, 144. https://doi.org/10.1016/j.ecoleng.2019.105712

Cardoso, C. P., Bazzo, J. H. B., Marinho, J. L. & Zukareli, C. 2021. Effect of seed vigor and sowing densities on the yield and physiological potential of wheat seeds. *Journal of Seed Science*, 43. http://dx.doi.org/10.1590/2317-1545v43241586

City of Cape Town. 2010. *City of Cape Town nature reserves: A network of amazing biodiversity*. City of Cape Town, Cape Town, South Africa.

City of Cape Town. 2018. *Biodiversity management branch restoration subsidiary plan: Tygerberg Nature Reserve*. Tygerberg Restoration Plan (Draft), Tygerberg Nature Reserve, Cape Town, South Africa.

City of Cape Town. 2019. *Draft Tygerberg district baseline and analysis report: state of the population and environment*. https://vdocuments.mx/draft-tygerberg-district-baseline-and-analysis-report-draft-tygerberg-district-baseline.html?page=1.

Cousins, S. R., Witkowski, T. F. & Esler, K. J. 2018. Influence of fire on critically endangered swartland shale renosterveld in the cape floristic region. *Applied Vegetation Science*, 21 (1) 144–155. https://doi.org/10.1111/avsc.12348

Cowell, C. R. 2007. Protocols for fynbos restoration. *Building a sustainable future: the role of botanic gardens.* Proceedings of the 3rd Global Botanic Gardens Congress, Wuhan, China. Retrieved from: https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Protocols+for+Fynbos+restoration&btnG=#d=gs_qabs&u=%23p%3DfKh4el7tj48J

Cowell, C. R. 2013. Investigating the most favourable seed establishment methods for restoring sand plain fynbos on old fields (master's thesis). Cape Peninsula University of Technology, Cape Town, South Africa, 1–138. Retrieved from: 198000472_Cowell_CR_2013.pdf

Du Plessis, N. S., Rebelo, A. J., Richardson, D. M. & Esler, K. J. 2021. Guiding restoration of riparian ecosystems degraded by plant invasions: Insights from a complex social-ecological system in the global south. *AMBIO A Journal of Environment and Society*, 51 (6), 1552–1568. http://dx.doi.org/10.1007/s13280-021-01691-y

Fox, J. & Weisberg, S. 2019. *An R Companion to Applied Regression*. Third edition, Chapter 6, 271–333. Sage, Thousand Oaks CA. Available: https://socialsciences.mcmaster.ca/jfox/Books/Companion/ Accessed on 05/11/2024

Gerrits, G. M., Waenink, R., Aradottir, A. L., Buisson, E., Dutoit, T., Ferreira, M. C.… & Wubs, E. R. J. 2023. Synthesis on the effectiveness of soil translocation for plant community restoration. *Journal of Applied Ecology*, 60, 714–724. https://doi.org/10.1111/1365-2664.14364

Hall, S. A., Holmes, P. M., Gaertner, M. & Esler, K. J. 2021. Active seed sowing can overcome constraints to passive restoration of a critically endangered vegetation type. *South African Journal of Botany*, 138, 249–261. https://doi.org/10.1016/j.sajb.2020.12.020

Holl, K. D., Luong, J. C. & Brancalion, P. H. S. 2022. Overcoming biotic homogenization in ecological restoration. *Trends in Ecology & Evolution*, 37 (9), 777–788. https://doi.org/10.1016/j.tree.2022.05.002

Holmes, P. M. 2002. Renosterveld restoration on cultivated lands at Eerste River, Western Cape: preliminary findings of a pilot study. *Unpublished report, Lafarge Quarries*.

Holmes, P. M. 2005. Results of a lucerne old-field restoration experiment at the Fynbos-Karoo interface. *South African Journal of Botany*, 71, 326–338.

Holmes, P. M., Esler, K. J., Gaertner, M., Geerts, S., Hall, S. A., Nsikani, M. M.... & Ruwanza, S. 2020a. Biological Invasions and Ecological Restoration in South Africa. In: van Wilgen, B., Measey, J., Richardson, D., Wilson, J., Zengeya, T. (eds) Biological Invasions in South Africa. *Invading Nature - Springer Series in Invasion Ecology*, 14, 665–700. Springer, Cham. https://doi.org/10.1007/978-3-030-32394-3_23

Holmes, P. M., Esler, K. J., Geerts, S., Ngwenya, D. K., Rebelo, A. G., Dorse, C.... & Nsikani, M. M. 2022. *Guidelines for Restoring Lowland Sand Fynbos Ecosystems*. Self-published. ISBN: 978-0-620-98765-3.

Horn, A., Krug, C. B., Newton, I. P. & Esler, K. J. 2011. Specific edge effects in highly endangered Swartland Shale Renosterveld in the Cape region. *Mediterranean Ecology*, 37 (2), 63–74.

Hull, A. C. J. 1972. Seeding rates and spacing for rangelands in south-eastern Idaho and northern Utah. *Journal of Range Management*, 23**,** 421–424.

Kassambara, A. 2023. *rstatix: Pipe-Friendly Framework for Basic Statistical Tests*. R package version 0.7.2, Available: https://CRAN.R-project.org/package=rstatix

Kilcher, M. R. & Heinrichs, D. H. 1968. Rates of seeding rambler alfalfa with dryland pasture grasses. *Journal of Range Management*, 21**,** 248–249.

Kirmer, A., Baasch, A. & Tischew, S. 2012. Sowing of low and high diversity seed mixtures in ecological restoration of surface mined-land. *Applied Vegetation Science*, 15, 198–207. https://doi.org/10.1111/j.1654-109X.2011.01156.x

Kravtsova, T. 2023. Fruit structure in amphicarpic annual *Gymnarrhena micrantha* (Asteraceae, gymnarrheneae) in relation to the species biology. *International journal of Plant Biology*, 14, 397–417. https://doi.org/10.3390/ijpb14020032

Krug, C. B. 2004. *Practical guidelines for the restoration of renosterveld*. University of Stellenbosch, Stellenbosch, South Africa. http://academic.sun.ac.za/consecol/RenosterveldRestorationProject/index.htm

Kulikowski A. J., Zahawi R. A., Werden L. K., Zhu K. N & Holl K. D. 2022. Restoration interventions mediate tropical tree recruitment dynamics over time. *Philosophical Transactions Royal Society B*, 378. https://doi.org/10.1098/rstb.2021.0077

Lehman, T. W. K., Esler, K. J. & Holmes, P. M. 2022. *Exploring the concept of applied nucleation as a restoration tool in a previously invaded Mediterranean climate vegetation type* (master's thesis). Stellenbosch University, Stellenbosch, South Africa. https://scholar.sun.ac.za/server/api/core/bitstreams/7d5d784d-8dab-42ab-8300-be9484b1be3a/content

Lenth, R. 2024. *emmeans: Estimated Marginal Means, aka Least-Squares Means*. R package version 1.10.1, Available: https://CRAN.R-project.org/package=emmeansResults: Accessed on 05/11/2024

Marcos-Filho, J. 2015. Seed vigor testing: an overview of the past, present and future perspective. *Scientia Agricola*, 72 (4), 363–374. https://www.scielo.br/scielo.php?script=sci_arttext&pid=S0103-90162015000400363

Mucina, L. & Rutherford, M. C. (eds). 2006. The vegetation of South Africa, Lesotho and Swaziland. *Strelitzia 19*. South African National Biodiversity Institute, Pretoria, South Africa.

Mukundamago, M. 2016. *Restoration of cape flats sand fynbos: the significance of pre-germination treatments and moisture regime* (master's thesis). University of Stellenbosch, Stellenbosch, South Africa.

Ngwenya D. K., Holmes, P. M., Geerts, S & Esler, K. J. 2023. Delaying a prescribed burn to scale up the restoration of alien-invaded lowland sand fynbos in South Africa. *Austral Ecology*, 49 (2), 1–23. https://doi.org/10.1111/aec.13469

Nsikani, M. M., van Wilgen, B. W., Bacher, S. & Gaertner, M. 2018. Re-establishment of *Protea repens* after clearing invasive *Acacia saligna*: consequences of soil legacy effects and a native nitrophilic weedy species. *South African Journal of Botany*, 116, 103–109. https://doi.org/10.1016/j.sajb.2018.02.396

Orrock, J. L., Brudvig, L. A., Damschen, E. I., Mattingly, W. B., Cruz, J., Veldman, J. W.… & Larsen-Grey, A. L. 2023. Long-term, large-scale experiment reveals the effects of seed limitation, climate, and anthropogenic disturbance on restoration of plant communities in a biodiversity hotspot. *Proceedings of the National Academy of Sciences*, 120 (7). https://doi.org/10.1073/pnas.2201943119

Ostoja, S. M., Schupp, E. W., Durham, S. & Klinger, R. 2013. Seed harvesting is influenced by associational effects in mixed seed neighbourhoods, not just by seed density. *Functional Ecology*, 27, 775–785. https://doi.org/10.1111/1365-2435.12091

Piaia, B. B., Rovedder, A., Giacomini, I. F., Felker, R. M., Stefanello, M. M. Hummel, R. & Schenato, R. B. 2022. Direct seeding for forest restoration in southern Brazil: influence of soil conditions and subtropical climate. *Revista Acta Ambiental Catarinense*, 20 (1), 01–12. https://doi.org/10.24021/raac.v20i1.6142

Power, S. C., Cramer, M. D., Verboom, G. A. & Chimpango, S. B. M. 2011. Legume seeders of the Cape Floristic Region inhabit more fertile soils than congeneric resprouters – sometimes. *Plant Ecology*, 212, 1979–1989. https://doi.org/10.1007/s11258-011-9958-3

Rashid, S., Rashid, K., Ganie, A. H., Nawchoo, I. A. & Khuroo, A. A. 2023. Seed ecology enlightens restoration of endemic species: A case study of *Actaea kashmiriana* from the Himalaya. *Ecological Engineering*, 187. https://doi.org/10.1016/j.ecoleng.2022.106880

Robinson, R., Beck, L. & Kettenring, K. M. 2023. The effects of native seed mix composition and sowing density on plant community reassembly in wetlands. *Ecosphere*, 15 (5). https://doi.org/10.1002/ecs2.4829

Ruwanza, S. 2017. Towards an integrated ecological restoration approach for abandoned agricultural fields in renosterveld, South Africa. *South African Journal of Science*, 113, 1–4. https://doi.org/http://doi.org/10.17159/sajs.2017/a0228.

R Core Team. 2024. R: *A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. Available: https://www.R-project.org/. Accessed on 05/11/2024

Traveset, A., Heleno, R. & Nogales, M. 2014. The ecology of seed dispersal. *The Ecology of Regeneration in Plant Communities*, 62–93, 3rd Edition (ed. R.S. Gallagher).

Turner, S.R., Steadman, K.J., Vlahos, S., Koch, J.M. & Dixon, K.W. 2013. Seed treatment optimizes benefits of seed bank storage for restoration-ready seeds: the feasibility of prestorage dormancy alleviation for mine-site revegetation. *Restoration Ecology*, 21, 186–192. https://doi.org/10.1111/j.1526-100X.2012.00879.x.

Wang, B. 2019. Density affects post-dispersal seed predation: evidence from a seed removal experiment of 62 species. *Integrative Zoology*, 15, 135–143. https://doi.org/10.1111/1749-4877.12421

Wickham, H. 2016. *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York.

Witt, C. V., Holmes, P. M. & Carrick, P. 2019. *Fynbos life: Swartland shale renosterveld.* Swartland Shale Renosterveld (fynboslife.com). Accessed on 13/05/2022

Vallentine, J. F. 1989. *Range Developments and Improvements*, New York, Academic Press, 254–263.

van Wilgen, B. W., Fill, J. M., Baard, J., Cheney C., Forsyth, A. T. & Kraaij, T. 2016. Historical costs and projected future scenarios for the management of invasive alien plants in protected areas in the Cape Floristic Region. *Biological Conservation*, 200, 168–177.

CHAPTER THREE: Improving restoration outcomes: species-specific approaches for restoring Swartland Shale Renosterveld

Abstract

Active restoration aims to restore the full complement of species, but despite sowing many species, often only a few species establish. In Swartland Shale Renosterveld (SSR), out of 41 species sown through active restoration, only 14 persisted. This study therefore aimed to test potential factors (seed viability, germination, pre-treatment methods) limiting germination and emergence for five "difficult-to-restore" species (*Dicerothamnus rhinocerotis*, *Podalyria sericea*, *Helichrysum dasyanthum*, *H. patulum,* and *H. teretifolium*) and create a restoration approach to enhance species establishment in SSR. The methodology encompassed (1) seed viability assessment via 2,3,5-tetraphenyltetrazolium chloride staining, (2) germination trials across various growth media, and (3) smoke treatment applications. Seed viability was below 50% for all species except *P. sericea* (73%), while germination was below 25% for all species, except for *H. patulum* (73%). Smoke treatment had no effect on the germination of *D. rhinocerotis* but increased *P. sericea* germination, and reduced germination of all three *Helichrysum* species. The study showed that *D. rhinocerotis* recruitment is limited by seed viability, *P. sericea* requires smoke as pre-treatment to break dormancy and for *Helichrysum* smoke exposure inhibits germination.

3.1 Introduction

Ecological restoration has been supported globally as an ecological strategy to enhance biodiversity and achieve functional ecosystems (Holl, Luong & Brancalion 2022). Recognized by the United Nations (UN) as a key global issue, ecological restoration was designated the focus of the 2021 – 2030 Decade to restore functional ecosystems (UN 2019; Nsikani et al. 2022). Ecosystems in southern Africa, particularly the Cape Floristic Region (CFR), have been severely threatened by habitat loss, degradation, and invasive alien species (Mukundamago 2016; Topp & Loos 2018; Nsikani et al. 2020; Orrock et al. 2023). Restoring threatened ecosystems is challenging, often failing to re-establish optimal plant diversity (Acosta et al. 2018; du Plessis et al. 2021). Thus, restoration efforts usually focus on building functionally resilient ecosystems first, with at least some plant diversity (Holmes et al. 2020b; du Plessis et al. 2021). Despite its importance for ecosystem function, restoring diverse plant communities in the CFR remains difficult (Cowell 2007, 2013; Mukundamago 2016; Ruwanza 2017; Nsikani 2018).

Renosterveld, a critically endangered CFR habitat (Topp & Loos 2019), harbours 214 threatened species, 25 of which are confined to the Swartland Renosterveld (Cousins 2017). Renosterveld has been rapidly declining due to habitat loss, degradation, and alien plant invasion (Witt et al. 2019). Active restoration is often advised for threatened ecosystems with depleted seed sources (Holmes et al. 2020a; 2020b); however, some species are particularly challenging to restore, as these do not easily establish from seed (Holmes et al. 2020a; Orrock et al. 2023). Such poor recruitment raises concerns for Swartland Shale Renosterveld (SSR) restoration efforts aimed at optimising plant diversity. Seed-based restoration in SSR has led to depauperate plant communities, with only 12 out of the 41 species sown, establishing successfully (Chapter 2). Some genera, such as *Helichrysum* recruited particularly poorly (Chapter 2). Afolayan, Meyer, and Leeuwner (1997) found higher germination in untreated *Helichrysum aureonitens* seeds than in smoke-treated ones, suggesting pre-treatments such as smoke treatment applied to all species may hinder restoration success. Seed-based restoration can be improved if practitioners understand species-specific seed ecology but for many renosterveld species this is unknown (Rashid et al., 2023). Therefore, ensuring successful germination and emergence is crucial for effective plant restoration (Pardini et al., 2017).

Successful restoration of many species, particularly local endemics, is often constrained by multiple interacting factors (Orrock et al. 2023). Key limitations include seed availability, competition with invasive plants, and predation, all of which significantly hinder ecological restoration in renosterveld ecosystems (Ruwanza 2017). Additionally, factors such as pollination, seed viability, storage conditions, pre-treatment methods, rainfall variability, and fire regimes may further impact restoration outcomes. Given these limitations, the first step in restoration efforts should be ensuring the use of viable, properly pre-treated seeds before addressing broader ecological constraints such as predation and competition by invasive plants. Seed viability is particularly influenced by pollination (Donaldson et al. 2002) and storage conditions (Rao et al. 2006; De Vitis et al. 2020), both of which can reduce the availability of viable seeds suitable for restoration. Furthermore, some species require specific pre-treatment methods to enhance germination (Brown et al. 2003; Brown & Botha 2004; Waller et al. 2015). For instance, some species may respond positively to heat or smoke treatments, whereas certain renosterveld endemics can germinate without additional treatment (Heelemann et al. 2013; Cowan & Anderson 2014; Cousins et al. 2018). Additionally, some species may germinate differently depending on the amount of sunlight received, soil conditions or growth media used for germination. Understanding these species-specific recruitment factors is essential for improving restoration success (Orrock et al. 2023; Rashid et al. 2023). To address these challenges, restoration efforts must adopt tailored strategies that account for species-specific emergence requirements (Orrock 2023).

This study aimed to develop a restoration approach for selected SSR species that are difficult to restore. Specifically, the study aimed to test seed viability, seed germination rates, and the influence of smoke treatment on five renosterveld species that had low or no recruitment during restoration (Chapter 2).

3.2 Methods

3.2.1 Seed collection

Seed collection was done at Tygerberg Nature Reserve (TNR), located at the Tygerberg Hills (-33.874835º S 18.596143º E). The reserve conserves the largest remainder of SSR vegetation (Horn et al. 2011). Swartland Shale Renosterveld is dominated by varieties of low to moderately tall shrubland, as well as open shrubland dominated by *Dicerothamnus rhinocerotis* (Mucina & Rutherford 2006). It harbours about 460 different plant species, with three species being restricted to the Tygerberg region and twelve species threatened with extinction (City of Cape Town 2019). Seed collection was done December 2021, then stored at TNR at room temperature (averaging 20°C–25°C), then retrieved during autumn of 2022 for conducting experiments.

3.2.2 Study species

The following species were selected for experiments to investigate the potential factors causing low recruitment in SSR: *D. rhinocerotis, Helichrysum dasyanthum, H. patulum, H. teretifolium,* and *Podalyria sericea*. These were selected from a list of potential species that showed low or no recruitment during restoration (Table S2). *Dicerothamnus rhinocerotis* dominates renosterveld (Mucina & Rutherford 2006) but shows low recruitment, appearing in only 4 of the 37 plots over three years, with fewer than five seedlings per plot. The selected *Helichrysum* species have either no recruitment (H. *dasyanthum* and *H. teretifolium*), or low recruitment (*H. patulum* recruiting 5–20 seedlings per plot on average), while for *P. sericea* recruitment was less than 10 seedlings per plot. In this study 1200 seeds per species were used across all experiments.

3.2.3 Seed viability

Viability tests and germination trials were conducted at the Millennium Seed Bank Partnership (MSBP) processing unit, based at Kirstenbosch National Botanical Garden (NBG). The tetrazolium (TZ) test procedures were conducted following methods recommended by Peters and Lanham (2002) and Afolayan et al. (1997). For *P. sericea*, seeds were imbibed in water for 24 hours to enhance water absorption prior to the tetrazolium chloride test due to its hard seed coat and then carefully dissected using a sterilised sharp blade. Seeds of all five species were then immersed in a liquid solution containing 1% 2,3,5-tetraphenyltetrazolium chloride and distilled water. The seeds were left immersed in the TZ solution overnight. A total of 25 seeds was used for each species and replicated four times ($n = 100$). Following the soaking period, seeds were carefully punctured with a needle under a stereomicroscope to avoid damaging the embryo (Afolayan et al. 1997). Viability was assessed with a stereomicroscope (ZEISS Stemi 305 stereo microscope) by quantifying embryos that exhibited a reddish coloration.

3.2.4 Seed germination

Germination trials were conducted for a period of three months. The experiment was set up using 9 cm transparent plastic petri dishes, using agar and filter paper separately as growth media (Rao et al. 2006). Petri dishes and all experimental tools were sterilized prior to use. Filter paper was moistened with distilled water and placed inside the petri dishes as the growth medium. The agar medium was prepared by dissolving 4 g of agar powder in 100 mL of distilled water, boiling the solution, and then cooling it before pouring it into the petri dishes. The petri dishes for both agar and filter paper growth media were randomly distributed inside the germination incubator set at room temperature (averaged 24°C) throughout the experiment. Optimal germination temperature ranges between 20°C to 30°C for many species (Rao et al. 2006), with room temperatures (23°C – 25°C) often preferred for germination. The experiment was exposed to a 14-hour photoperiod and 10 hours of darkness, following the standard operation procedures of the MSBP (South African National Biodiversity Institute n.d). Three replicates of 16 seeds per petri dish were used for each treatment. The design was completely randomised with growth media and species.

3.2.5 Influence of smoke treatment on germination and seedling emergence

The experiment for smoke treatments was conducted over a six-month period at the Kirstenbosch NBG. The greenhouse for this experiment comprised a light-penetrating rooftop made with polycarbonate roofing. The surface area inside the greenhouse is a precast concrete floor, which allows water to run off towards designated catchment areas. The computerised temperature system was set to be fluctuating between 12°C minimum and 27°C maximum with a relative humidity of 57%, following the standard propagation procedures applied for germinating (South African National Biodiversity Institute n.d). The raised heating benches on which germination trays were placed, were set at 23°C throughout the experiment period as to support optimal soil temperature conditions during the cold season. Irrigation was done daily (~ 400ml – 800ml per tray) using a manual misting system, with intervals based on the growth media's moisture content to keep it moist but not saturated.

The seed smoking procedure involved preparing species-specific trays, labelled with the species name and smoking date. Tiny seeds were contained in small envelopes to prevent loss during the process, with the envelopes left open to ensure smoke access. These envelopes were placed inside an enclosed plastic tunnel. A mixture of dry and green fynbos plant material was ignited in a metal drum, and the resulting smoke was directed through steel pipes into the tunnel containing the seeds. The seed trays were exposed to smoke for approximately four hours.

Both smoked and non-smoked seeds were sown on the same date under identical environmental conditions. The growth medium consisted of Kirstenbosch NBG standard nursery soil, formulated with six parts well-decomposed compost, two parts well-aged bark (5–12 mm), and one part fine white sand. The growth medium was initially sifted to remove coarse particles that could adversely affect seedling emergence. The removed coarse particles were placed at the bottom of the trays to enhance drainage and aeration on the internal surfaces of the trays. Flat black plastic reusable seed trays (15 cm x 20 cm x 6 cm) were utilized for *D. rhinocerotis* and *Helichrysum*. The larger *Podalyria sericea* seeds were sown in larger trays (35 cm x 48 cm x 8 cm). The experiment involved two treatments, each with five replicates. For each species, half of the seeds (n = 100) were subjected to seed smoking, while the other half remained untreated. All sowed trays were randomly distributed within the greenhouse to avoid biasness on the sunlight and water received by each treatment. Germination count was done by manually counting cotyledons (first leaf set emerging) from each replicate on a weekly basis. Weekly counts were conducted to account for seedlings that might die back after germination, ensuring that final seedling emergence rather than emergence rate over time was recorded.

3.2.6 Statical analyses

To determine the effect of seed viability between species, a generalized linear model (GLM) with a Poisson distribution and log link function was applied to the data. The main effects were calculated using the '*car*' package (Fox and Weisberg 2019) through a chi-squared test to test for association between the variables. All assumptions for a Poisson generalized linear model were met.

To investigate the relationship between germination percentages for the five different species and two growth media, a GLM with a Poisson distribution and log link function was used. Main effects were calculated using the '*car*' package using a chi-squared test to test for an association between germination percentages, species, and two growth media. The pairwise post-hoc analysis using a z-distribution for comparing the interaction between species and growth media, along with its effect on germination percentage, was calculated using the '*emmeans*' package (Length 2024).

For the relationship between seedling emergence percentages for five different species and pre-treatment methods, a GLM with a Poisson distribution and log link function was applied to the data. Main effects for the association between the variables were determined using the '*car*' package through a chi-squared test. The interaction between species and pre-treatment methods, along with its impact on seedling emergence percentages, was pairwise analysed using the '*emmeans*' package. All statistical analyses were performed using R v 4.4.0 (R Core Team 2024), and graphics were generated using the packages '*ggplot2*' (Wickham 2016) and '*ggsignif*' (Ahlmann-Eltze & Patil 2021).

3.3 Results

3.3.1 Seed viability

The species ($X^2 = 410.50$, df = 4, $p < 0.001$) had a significant influence on seed viability. Seed viability was low across all species with *Podalyria sericea* showing a significantly higher viability at 73% (Fig. 3.1). In contrast, *Dicerothamnus rhinocerotis* had a significantly lower viability at only 2%. Within the genus *Helichrysum*, *H. dasyanthum* showed significantly higher viability (43%) than *H. teretifolium* (26%), while *H. patulum* (34%) did not differ significantly from either (Figure 3.1).

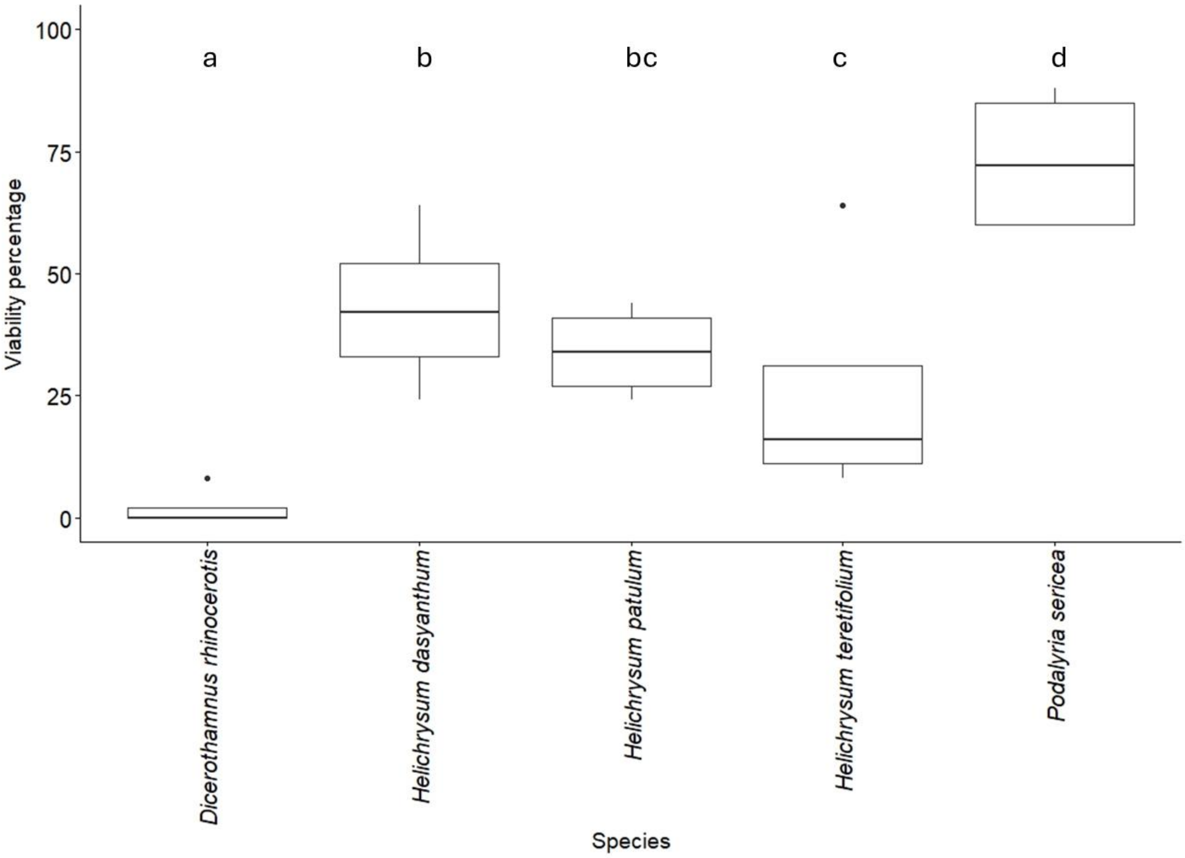

Figure 3.1: Seed viability (in percentage) comparison for the five study species. The box represents the interquartile range (IQR), the middle line represents the median, and the whiskers represent 1.5x IQR or range; the points represent outliers. The significant difference between species is indicated by letters (same letters indicate no significant difference).

3.3.2 Seed germination

Germination rates differed significantly between growth media for *D. rhinocerotis*, *H. dasyanthum*, and *P. sericea* (Figure 3.2). *Dicerothamnus rhinocerotis* exhibited low germination overall, with a significantly higher rate of 9% on filter paper compared to 2% on agar (Figure 3.2). Conversely, agar promoted higher germination in *H. dasyanthum* (22% vs. 2% on filter paper) and *P. sericea* (10% vs. 4%) (Figure 3.2). No significant differences were observed for *H. patulum* and *H. teretifolium* ($p > 0.05$). Amongst all species, *H. patulum* had the highest germination rates, with 73% on filter paper and 63% on agar, while *H. teretifolium* showed low germination (19% on filter paper and 17% on agar) (Figure 3.2).

Pairwise comparisons on agar revealed significant differences amongst species ($X^2 = 583.31$, df = 4, $p < 0.001$) except for *H. dasyanthum* vs. *H. teretifolium* ($p = 0.646$) and *H. teretifolium* vs. *P. sericea* (*p* = 0.195). *Helichrysum patulum* had significantly higher germination than all other species on agar (Figure 3.2). On filter paper, all species comparisons were significant (*p* < 0.05) except for *P. sericea* vs. *D. rhinocerotis* (*p* = 0.149) and *P. sericea* vs. *H. dasyanthum* (*p* = 0.519). Again, *H. patulum* showed significantly higher germination than the other species (Figure 3.2).

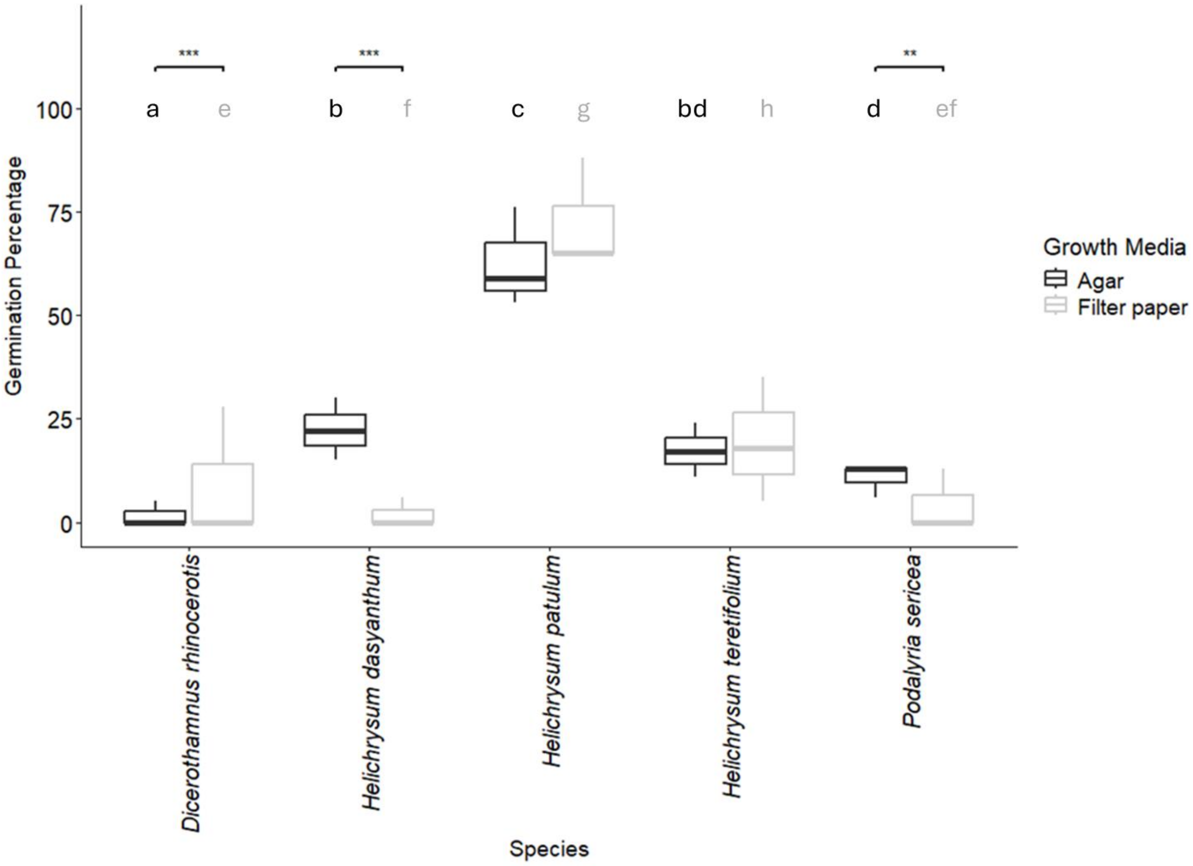

Figure 3.2: Germination rates (in percentage) for the five study species for two different growth media. The box represents the interquartile range (IQR), the middle line represents the median and the whiskers represent 1.5x IQR or range; the points represent outliers. The significant difference between species is indicated by letters (same letters indicate no significant difference) with two different sets of letters for growth media. The significant difference between growth media for each species is indicated by the bars and asterisks, Significance level: * (P < 0.05), ** (P < 0.01) and *** (P < 0.001).

3.3.3 Influence of smoke treatment on germination and seedling emergence

Seedling emergence was low across all species (Figure 3.3). Significant differences were observed between smoked and non-smoked treatments ($X^2 = 21.98$, df = 1, $p < 0.001$) for all species except *D. rhinocerotis* (Figure 3.3). All *Helichrysum* species showed higher emergence percentages with non-smoked seeds, while *P. sericea* had higher emergence with smoked seeds

(Figure 3.3). For *D. rhinocerotis*, emergence averaged 2% in the smoked treatment, with no germination in the non-smoked treatment (Figure 3.3).

For comparisons between species within the non-smoked treatment, *D. rhinocerotis* had zero seedling emergence (Figure 3.3); hence, statistical significance was not tested for this species. The species *H. patulum* had a significantly higher seedling emergence percentage than *H. dasyanthum, H. teretifolium,* and *P. sericea* (X^2 = 74.41, df = 4, $p < 0.05$). There was no significant difference between *H. dasyanthum*, *H. teretifolium,* and *P. sericea* (Figure 3.3).

For smoked treatment comparisons between species, there was no significant difference between *D. rhinocerotis* and all other species ($p > 0.05$), except for *P. sericea* ($p < 0.001$) which had a significantly higher seedling emergence (Figure 3.3). There was no significant difference between the *Helichrysum* species (Figure 3.3).

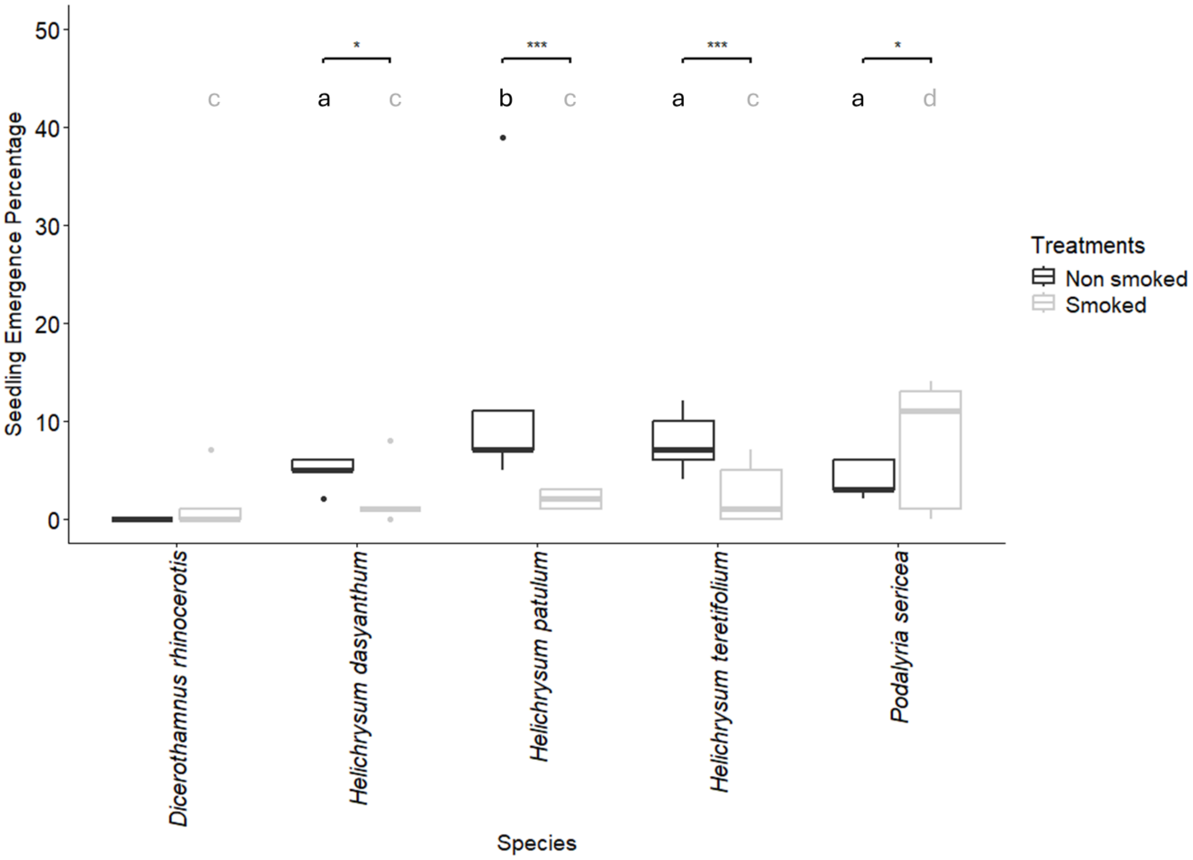

Figure 3.3: Seedling emergence (in percentage) for the five study species for non-smoked and smoked treatments. The box represents the interquartile range (IQR), the middle line represents the median, and the whiskers represent 1.5x IQR or range; the points represent outliers. The significant difference between species is indicated by letters (same letters indicate no significant difference) with two different

sets of letters for growth media. The significant difference between treatments for each species is indicated by the bars and asterisks, Significance level: * ($P < 0.05$), ** ($P < 0.01$) and *** ($P < 0.001$).

3.4 Discussion

Seed viability

This chapter explored whether seed viability, germination or smoke treatment could explain the low recruitment of selected Swartland Shale Renosterveld (SSR) species. Except for *Podalyria sericea*, seed viability was low and ranged from 2–43% (Figure 3.1). The seed viability of *P. sericea* was significantly higher at 73%, but even this is just below the recommended thresholds of 75% for wild plants (Rao et al. 2006) and the 80% suggested for restoration projects (Frischie et al. 2020; Pedrini & Dixon 2020). Similar viability levels were reported by Waller et al. (2015), who recorded a mean viability of 79.0 ± 5.97% for *P. sericea* in the restoration of Peninsula Shale Renosterveld. The *Helichrysum* species (*H. patulum*, *H. dasyanthum*, and *H. teretifolium*) had a seed viability below 50% (Figure 3.1), highlighting the need to improve viability of the genus. Waller et al. (2015) achieved a seed viability above 80% on *H. cymosum* subsp. *cymosum* and *H. patulum*. Testing seed viability can aid in improving quality of seeds collected for seed-based restoration. This ensures that collected seed lots contain a high proportion of living seeds with the ability to germinate. Viability testing allows practitioners to distinguish between live and dead seeds, improving estimates of effective sowing density and avoiding the use of non-viable seeds (Gosling 2003; International Seed Testing Association 2020). Identifying low-quality or aged seed lots can help to refine collection strategies, prioritize fresh or high-viability sources, and adjust storage or pretreatment methods to maintain quality (Merritt & Dixon 2011). This may collectively improve cost-effectiveness, enhance establishment rates, and ultimately increase the likelihood of restoring diverse, resilient plant communities.

Dicerothamnus rhinocerotis resulted in 2% viability, suggesting that seed viability is at least one of the reasons for low recruitment of this species during restoration activities. Seed viability is influenced by the period and conditions of storage (De Vitis et al. 2020). Pedrini & Dixon (2020) recommended 15% relative humidity and 15°C for short- to medium-term storage, i.e., 4–6 months. The seeds used for this study were stored at uncontrolled, often high temperatures (20–25°C), which might have contributed to low viability (Rao et al. 2006; De Vitis et al. 2020). The potential influence of seed storage on seed viability in this study remains uncertain and warrants investigation; however, it is plausible given that *D. rhinocerotis* seeds persist in soil seed banks for extended periods and therefore storage should be possible under optimum conditions (Cousins et al. 2018). Other than seed storage, *D. rhinocerotis* is a dioecious shrub and cross-pollination activities may be limited for such species in fragmented or degraded habitats (Donaldson et al. 2002), which can reduce the production of viable seeds. Therefore, understanding the seed ecology and reproductive biology of *D. rhinocerotis* is crucial for restoration efforts in SSR and other threatened renosterveld vegetation types.

Seed germination

A 50% germination rate is suggested as the minimum threshold for adequate germinability (Pedrini & Dixon 2020). Germination was below 50% for all species except *H. patulum* (Figure 3.2). *Podalyria sericea* demonstrated low overall germination (Figure 3.2), which may have been constrained by dormancy mechanisms or sensitivity to germination and emergence conditions. Even though germination was low, *P. sericea* germinated better on agar than filter paper, suggesting that growth media might influence germination outcomes. According to Rao et al. (2006), agar stays moist for up to one month and is particularly suitable as a growth media for germinating dormant seeds, while filter paper is recommended for small, quick-germinating seeds. This highlights the need for suitable growth media and suggests using moisture-holding substrates for restoring dormant-seed species, avoiding reliance on costly, labour-intensive irrigation. Substrates such as hydrogel and coir effectively provide moisture over time (Sarvas et al. 2007; Crous 2017; Kumar et al. 2020; Mariotti et al. 2020), which can enhance germination and emergence in restoration, since agar is only suitable for germinating seedlings in the lab, not for field planting. However, while both hydrogel and coir can boost short-term establishment, their long-term effects on native soil and plant community composition should be carefully considered in restoration planning.

There were varied germination outcomes across the genus *Helichrysum* (Figure 3.2), with *H. patulum* germinating significantly higher (73% vs. 63%) than *H. dasyanthum* (22% vs. 2%) and *H. teretifolium* (19% vs. 17%). Previous SSR restoration outcomes also revealed *H. patulum* as the species with better restoration potential, since it was the only *Helichrysum* species that emerged inside restoration plots (Chapter 2). Waller et al. (2017) achieved comparable results to this study, with *H. cymosum* subsp. *cymosum* and *H. patulum* obtaining above 70% germination, hinting at the high restoration potential of these species. *Helichrysum patulum* and *H. teretifolium* germinated consistently across different media types, suggesting that growth media had no influence on germination. For *H. dasyanthum*, germination rates significantly differed between growth media, with a positive response under agar. The positive germination response under agar may be reflecting sensitivity to moisture availability or growth media characteristics (Muñoz-Rojas et al. 2016), since agar holds moisture for a longer period (Rao et al. 2006). Other studies on fynbos species have noted that substrate type and moisture levels can drastically influence germination success, suggesting that fine-tuning growth conditions for SSR restoration may improve germination for *Helichrysum* species (Holmes & Richardson, 1999). *Dicerothamnus rhinocerotis* has resulted to low germination outcomes (Figure 3.2), likely due to the low seed viability achieved (2%).

Influence of smoke treatment on germination and seedling emergence

Seedling emergence through smoke and non-smoked treatment was below 15% across all species (Figure 3.3). Seedling emergence was lower than germination across species, likely due to soil conditions, as germination occurred in incubation while emergence occurred in soil. The seedling emergence of *P. sericea* exhibited a significant positive response to smoke treatment ($p < 0.05$). This finding aligns with other studies that suggest smoke can stimulate germination in some species adapted to fire-prone environments (Curtis et al. 2024). Heat and smoke treatments, either through hot water, smoke water, or direct exposure to aerosol smoke, have been successful in triggering germination in a range of fynbos legumes (Brown et al. 2003; Brown & Botha 2004), including *P. sericea* (van Staden et al. 2000). This study did not account for heat scarification to overcome seed dormancy prior to germination tests, which could have improved germination outcomes of this species. For instance, high germination and emergence of *P. sericea* was achieved following hot-water and smoke treatment as the pre-treatment methods (Waller et al. 2015). Comparatively, studies on other species in the Fabaceae family, such as *Aspalathus* and some *Mimosa* species, have also demonstrated enhanced germination and emergence after exposure to heat and smoke, suggesting that this trait is common in fire-adapted legumes (Zirondi et al. 2019; Curtis et al. 2024).

The three *Helichrysum* species showed markedly better germination and emergence under non-smoked treatment (Figure 3.3). Comparable results were obtained by Afolayan et al. (1997) on the germination of *Helichrysum aureonitens* using smoked seeds and non-smoked seeds, which exhibited a better germination on non-smoked seeds. *Helichrysum patulum* displayed the most significant difference, with smoke treatments yielding low emergence (1% to 3%) which increased to 39% without smoke treatments. These results indicated that *Helichrysum* species fare better in non-smoked conditions, hinting at a possible inhibitory effect of smoke on their germination. Smoke treatment has been part of the standard procedures for all seeds to be used for SSR restoration (Chapter 2), which might have been the constraint to the restoration success of these *Helichrysum* species. With about 20 *Helichrysum* species found in renosterveld (Rutherford et al., 2006), this might be applicable to other *Helichrysum* species, but also species from other genera. Certain SSR species (e.g. *Helichrysum teretifolium* and *Senecio pubigerus*) at TNR showed no response to smoke treatment in prior restoration efforts, and these warrant further study (Heelemann et al. 2013). Enhanced recruitment of these species may be achieved by omitting smoke treatment.

Smoke-treatment has no influence on germination and emergence of *D. rhinocerotis* (Figure 3.3). In contrast, Cousins et al. (2018) found a positive response in *D. rhinocerotis* to smoke-treated seeds, with germination increasing 2.5 times compared to untreated seeds. Additionally, Simons et al. (2017) noted higher recruitment of *D. rhinocerotis* in burnt areas than in unburned areas, hinting at possible influence by heat and smoke. The extremely low viability, germination, and emergence of *D. rhinocerotis* presents a significant barrier, highlighting the need to study the reasons for low viability, such as seed collection methods and storage, or considering a heat pretreatment. Alternatively, restoration strategies such as vegetative propagation may be necessary when there is a need to restore this species (Turner et al. 2018; Holmes et al. 2020a).

Implications for seed-based restoration

To meet the global restoration targets, new restoration approaches and techniques for a diversity of native plant taxa are needed (Frischie et al. 2020). This study revealed that for some species in SSR restoration, seeds that are sown have a low viability, while other species may be inhibited by smoke treatment. Additionally, seeds were stored in air-permeable bags under elevated humidity and temperature conditions, exceeding recommended levels (i.e. 15% relative humidity and 15°C (Pedrini & Dixon 2020)), leading to viability loss. To improve that, seeds should be subjected to seed quality tests before storage and sowing (Pedrini & Dixon 2020). Furthermore, viable seeds should be packaged in dry, airtight containers and stored under the storage facility with recommended conditions for seed storage. Further research is needed to refine species-specific pre-treatments for breaking seed dormancy. For species which lacks adequate seed viability and not limited by dormancy, alternative reproduction and restoration approaches, i.e. propagation by cuttings, are recommended (Turner et al. 2020).

The varied outcomes of this study underscore the importance of species-specific restoration strategies to address germination and emergence constraints in native species (Merritt & Dixon 2011; Araujo & Perez 2022). Beyond the factors tested, species richness in SSR restoration is influenced by predation, seed recalcitrance, and competition from invasive alien plants. Some species in SSR, particularly geophytes, produce recalcitrant seeds that lose viability quickly when desiccated, complicating storage and restoration efforts. Abiotic factors, such as rainfall variability (Byun et al. 2023), further limit restoration success in SSR. These findings highlight the need for further research to address these challenges and enhance renosterveld restoration outcomes.

3.5 Conclusion

The findings of this study showed that factors limiting restoration success on selected species act independently, necessitating species-specific interventions to improve restoration success. For *D. rhinocerotis*, seed viability was identified as a key limitation, alongside other untested factors. The species *P. sericea* had a relatively high seed viability and a higher seedling emergence under smoke treatment, but with limited germination. This suggests that *P. sericea* may require pretreatment methods such as heat scarification and smoke to enhance its germination for restoration. In contrast, *Helichrysum* species proved to respond positively when germinated under controlled incubation conditions. Additionally, *Helichrysum* species exhibited better seedling emergence under non-smoked conditions, suggesting that smoke exposure may inhibit their emergence. Smoke treatment is suitable for certain species in SSR, particularly those that need fire to break the dormancy. By conducting seed viability tests and tailoring seed treatments to species requirements, restoration practitioners can increase restoration success relatively easy. Species can be grouped in those requiring smoke, heat and smoke, or no treatment at all. This together with appropriate seed viability and seed storage will increase species richness, but also abundance when actively restoring SSR.

References

Abedi, M., Zaki, E., Erfanzadeh, R. & Naqinezhad, A. 2018. Germination patterns of the scrublands in response to smoke: the role of functional groups and the effect of smoke treatment method. *South African Journal of Botany,* 115, 231–236. http://dx.doi.org/10.1016/j.sajb.2017.03.010

Acosta, A. L., de Carvalho, F. A. G., Leite, M. S., Saraiva, A. M. & Metzger, J. P. W. 2018. Gaps and limitations in the use of restoration scenarios: a review. *Restoration ecology*, 26 (6), 1108–1119. https://doi.org/10.1111/rec.12882

Afolayan, A. J., Meyer J. J. M. & Leeuwner D. V. 1997. Germination in *Helichrysum aureonitells* (Asteraceae): Effects of temperature, light, gibberellic acid, scarification and smoke extract. *South African Journal of Botany*, 63 (1), 22–24.

Ahlmann-Eltze, C. & Patil, I. 2021. ggsignif: R Package for Displaying Significance Brackets for 'ggplot2'. *PsyArxiv.* https://doi.org/10.31234/osf.io/7awm6

Araujo, M. E. R. & Perez, D. R. 2022. From seed germination to established seedlings: a comparative evaluation in five shrub species and implications for seed-based restoration in arid lands. *Restoration Ecology*, 31 (6). https://doi.org/10.1111/rec.13862

Brown, N. A. C., van Staden, J., Daws, M. I. & Johnson, T. 2003. Patterns in the seed germination response to smoke in plants from the Cape Floristic Region, South Africa. *South African Journal of Botany*, 69 (4), 514–525. https://doi.org/10.1016/S0254-6299(15)30289-1

Brown, N. A. C. & Botha, P. A. 2004. Smoke seed germination studies and a guide to seed propagation of plants from the major families of the Cape Floristic Region, South Africa. *South African Journal of Botany*, 70 (4), 559–581. https://doi.org/10.1016/S0254-6299(15)30194-0

Cochrane, A., Hoyle, G. L., Yates, C. J. & Wood, J. 2020. Direct seeding for ecological restoration: Overcoming barriers to seedling establishment. *Ecological Restoration*, 38 (3), 166–178.

Cowell, C. R. 2013. Investigating the most favourable seed establishment methods for restoring sand plain fynbos on old fields (master's thesis). Cape Peninsula University of Technology, Cape Town, South Africa, 1–138. Retrieved from: 198000472_Cowell_CR_2013.pdf

Crous, J. W. 2017. Use of hydrogels in the planting of industrial wood plantations. *Southern Forests*, 79 (3), 197–213.

Curtis, O. E., Bond, W. J. & Chimphango, S. 2024. Diversity and fire responses in renosterveld, the forgotten relation of fynbos, in southernmost Africa. *Journal of Arid Environments*, 225. https://doi.org/10.1016/j.jaridenv.2024.105261

Donaldson, J. S., Nänni, I., Zachariades, C. & Kemper, J. 2002. Effects of habitat fragmentation on pollinator diversity and plant reproductive success in renosterveld shrublands of South Africa. *Conservation Biology*, 16 (5), 1267–1276.

De Vitis, M., Hay, F. R., Dickie, J. B., Trivedi, C., Choi, J. & Fiegener, R. 2020. Seed storage: maintaining seed viability and vigor for restoration use. *Restoration ecology*, 28 (S3), S249–S255. https://doi.org/10.1111/rec.13174

Du Plessis, N. S., Rebelo, A. J., Richardson, D. M. & Esler, K. J. 2021. Guiding restoration of riparian ecosystems degraded by plant invasions: Insights from a complex social-ecological system in the global south. *AMBIO A Journal of Environment and Society*, 51, 1552–1568. https://doi.org/10.1007/s13280-021-01691-y

Frischie, S., Miller, A. L., Pedrini, S. & Kildisheva, O. A. 2020. Ensuring seed quality in ecological restoration: native seed cleaning and testing. *Restoration Ecology*, 28 (S3), S239–S248. https://doi.org/10.1111/rec.13217

Fox, J. & Weisberg, S. 2019. *An R Companion to Applied Regression*. Third edition. Sage, Thousand Oaks CA. Available: https://socialsciences.mcmaster.ca/jfox/Books/Companion/. Accessed on 05/11/2024

Gosling, P. 2003. *Viability testing*. In R. D. Smith, J. B. Dickie, S. H. Linington, H. W. Pritchard & R. J. Probert (Eds.), Seed conservation: turning science into practice, 445–481. Royal Botanic Gardens, Kew.

Heelemann, S., Krug, C. B., Esler, K. J., Reisch, C. & Poschlod, P. 2013. Soil seed banks of remnant and degraded Swartland Shale Renosterveld. *Applied Vegetation Science*, 16, 585–597. https://doi.org/10.1111/avsc.12026

Holl, K. D., Luong, J. C. & Brancalion, P. H. S. 2022. Overcoming biotic homogenization in ecological restoration. *Trends in Ecology & Evolution*, 37 (9), 777–788. https://doi.org/10.1016/j.tree.2022.05.002

Holmes, P. M. & Newton, R. 2004. Patterns of seed persistence in South African Fynbos. *Plant Ecology*, 172 (1), 143–158. https://doi.org/10.1023/B:VEGE.0000026035.73496.34

Holmes, P. M. & Richardson, D. M. 1999. Protocols for restoration based on recruitment dynamics, community structure, and ecosystem function: perspectives from South African fynbos. *Restoration Ecology*, 7 (3), 215–230. https://doi.org/10.1046/j.1526-100X.1999.72015.x

Holmes, P. M., Esler, K. J., Gaertner, M., Geerts, S., Hall, S. A., Nsikani, M. M.… & Ruwanza, S. 2020a. Biological invasions and ecological restoration in South Africa. *Biological Invasions in South Africa*, 665–700. https://doi.org/10.1007/978-3-030-32394-3_23

Holmes, P. M., Esler, K. J., Gaertner, M., van Wilgen, B. W. & Richardson, D. M. 2020b. Ecological restoration of ecosystems degraded by invasive alien plants in South African Fynbos: Is spontaneous succession a viable strategy?, *Transactions of the Royal Society of South Africa*, 75 (2), 111–139. https://doi.org/10.1080/0035919X.2020.1781291

International Seed Testing Association. 2020. *International rules for seed testing 2020*. Bassersdorf, Switzerland: ISTA.

Kumar, R., Yadav, S., Singh, V., Kumar, M. & Kumar, M. 2020. Hydrogel and its effect on soil moisture status and plant growth: A review. *Journal of Pharmacognosy and Phytochemistry*, 9 (3), 1746–1753.

Lenth, R. 2024. *emmeans: Estimated Marginal Means, aka Least-Squares Means*. R package version 1.10.1, Available: https://CRAN.R-project.org/package=emmeansResults: Accessed on 05/11/2024

Mariotti, B., Martini, S., Raddi, S., Tani, A., Jacobs, D. F., Oliet, J. A. & Maltoni, A. 2020. Coconut coir as a sustainable nursery growing media for seedling production of the ecologically diverse Quercus species. *Forests*, 11 (5), 522.

Merritt, D. J. & Dixon, K. W. 2011. Restoration seed banks—a matter of scale. *Science*, 332 (6028), 424–425. https://doi.org/10.1126/science.1203083

Merritt, D. J., Turner, S. R., Clarke, S. & Dixon, K. W. 2010. Seed dormancy and germination stimulation. In *Seed Conservation: Turning Science into Practice*, 215–249. Royal Botanic Gardens, Kew.

Moncrieff, G. R. 2021. Locating and dating land cover change events in the renosterveld, a critically endangered shrubland ecosystem. *Remote Sensing*, 13, 834. https://dx.doi.org/10.3390/rs13050834

Mucina, L. & Rutherford, M. C. (eds). 2006. The vegetation of South Africa, Lesotho and Swaziland. *Strelitzia 19*. South African National Biodiversity Institute, Pretoria, South Africa.

Mukundamago, M. 2016. *Restoration of cape flats sand fynbos: the significance of pre-germination treatments and moisture regime* (master's thesis). University of Stellenbosch, Stellenbosch, South Africa.

Muñoz-Rojas, M., Erickson, T. E., Martini, D. C., Dixon, K. W. & Merritt, D. J. 2016. Climate and soil factors influencing seedling recruitment of plant species used for dryland restoration. *SOIL*, 2, 287–298. https://doi.org/10.5194/soil-2-287-2016

Navie, S. C., Cowie, I. D. & Cook, G. D. 2017. Seed viability in tropical savanna species: Implications for revegetation. *Ecological Management & Restoration*, 18 (1), 66–72.

Nsikani, M. M., Anderson, P., Bouragaoui, Z., Geerts, S., Gornish, E. S., Kairo, J. G.… & Peer, N. 2022. UN decade on ecosystem restoration: key considerations for Africa. *Restoration Ecology*, 31 (3). https://doi.org/10.1111/rec.13699

Nsikani, M. M., Geerts, S., Ruwanza, S. & Richardson, D. M. 2020. Secondary invasion and weedy native species dominance after clearing invasive alien plants in South Africa: Status quo and prognosis. *South African Journal of Botany*, 132, 338–345. https://doi.org/10.1016/j.sajb.2020.05.009

Nsikani, M. M., van Wilgen, B. W., Bacher, S. & Gaertner, M. 2018. Re-establishment of *Protea repens* after clearing invasive *Acacia saligna*: consequences of soil legacy effects and a native nitrophilic weedy species. *South African Journal of Botany*, 116, 103–109. https://doi.org/10.1016/j.sajb.2018.02.396

Orrock, J. L., Brudvig, L. A., Damschen, E. I., Mattingly, W. B., Cruz, J., Veldman, J. W.… & Larsen-Grey, A. L. 2023. Long-term, large-scale experiment reveals the effects of seed limitation, climate, and anthropogenic disturbance on restoration of plant communities in a biodiversity hotspot. *Proceedings of the National Academy of Sciences*, 120 (7). https://doi.org/10.1073/pnas.2201943119

Pan American Seed. 2022. *Best practices for storing and handling seed*. Pan American Seed, West Chicago, United States of America. Seed-Storage-Best-Practices-BallSeed-TechOnDemand-PanAmericanSeed.pdf

Pardini, E. A., Patten, M. V. & Knight, T. M. 2017. Effects of seed density and proximity to refuge habitat on seed predation rates for a rare and a common *Lupinus* species. *American Journal of Botany*, 104 (3), 389–398. http://www.amjbot.org/

Pedrini, S. & Dixon, K. W. 2020. International principles and standards for native seeds in ecological restoration. *Restoration Ecology*, 28 (S3), S286–S303. https://doi.org/10.1111/rec.13155

Peters, J. & Lanham, B. 2002. *Tetrazolium testing handbook: contribution no. 29 to the handbook on seed testing*. The Association, Open Library.

Rao, N. K., Hanson, J., Dulloo, M. E., Ghosh, K., Nowell, D. & Larinde, M. 2006. *Manual of seed handling in genebanks*. Handbooks for Genebanks No. 8. Bioversity International, Rome, Italy.

Rashid, S., Rashid, K., Ganie, A. H., Nawchoo, I. A. & Khuroo, A. A. 2023. Seed ecology enlightens restoration of endemic species: A case study of *Actaea kashmiriana* from the Himalaya. *Ecological Engineering*, 187. https://doi.org/10.1016/j.ecoleng.2022.106880

Rutherford, M. C., Mucina, L. & Powrie, L. W. 2006 (Reprint 2011). Biomes and bioregions of southern Africa. In: L. Mucina, L. and Rutherford, M.C. (eds), *The vegetation of South Africa, Lesotho and Swaziland. Strelitzia 19*. South African National Biodiversity Institute, Pretoria.

Ruwanza, S. 2017. Towards an integrated ecological restoration approach for abandoned agricultural fields in renosterveld, South Africa. *South African Journal of Science*, 113, 1–4. https://doi.org/http://doi.org/10.17159/sajs.2017/a0228.

R Core Team 2024. R: *A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. Available: https://www.R-project.org/. Accessed on 05/11/2024

SANBI (South African National Biodiversity Institute). n.d. *Millennium Seed Bank Partnership*. Unpublished procedures.

Sarvaš, M., Pavlenda, P. & Takácová, E. 2007. Effect of hydrogel application on survival and growth of pine seedlings in reclamations. *Journal of Forest Science*, 53 (5), 204–209.

Schutte-Vlok, A. L. & Raimondo, D. 2012. *Podalyria sericea* (Andrews) R.Br. ex Aiton f. National Assessment: Red List of South African Plants version. Accessed on 2025/02/03.
Topp, E. N. & Loos, J. 2019. Fragmented landscape, fragmented knowledge: a synthesis of renosterveld ecology and conservation. *Environmental Conservation*, 46, 171–179. https://doi.org/10.1017/S0376892918000498

Turner, S. R., Merritt, D. J. & Clarke, S. 2018. Seed pre-treatments for overcoming dormancy. *Ecological Restoration*, 36 (4), 307–314.

United Nations Environment Assembly (UNEA) .2019. Resolution 73/284: United Nations Decade on Ecosystem Restoration (2021–2030). https://undocs.org/A/RES/73/284

Van Staden, J., Brown, N. A. C., Jäger, A. K. & Johnson, T. A. 2000. Smoke as a germination cue. *Plant Species Biology*, 15, 167–178.

Waller, P. A., Anderson, P. M., Holmes, P. M. & Newton, R. J. 2015. Developing a species selection index for seed-based ecological restoration in Peninsula Shale Renosterveld, Cape

Town. *South African Journal of Botany*, 99, 62–68. http://dx.doi.org/10.1016/j.sajb.2015.03.189

Wickham, H. 2016. *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York.

Witt, C. V., Holmes, P. M. & Carrick, P. 2019. *Fynbos life: Swartland shale renosterveld.* Swartland Shale Renosterveld (fynboslife.com). Accessed on 13/05/2022.

Zirondi, H. L., Silveira, F. A. O. & Fidelis, A. 2019. Fire effects on seed germination: heat shock and smoke on permeable vs impermeable seed coats. *Flora*, 253, 98–106. https://doi.org/10.1016/j.flora.2019.03.007

CHAPTER FOUR: General conclusion and recommendations

4.1 Summary

Active restoration is often a suitable strategy to restore highly degraded ecosystems (Ruwanza et al. 2013; Krupek et al. 2016; Holmes et al. 2020). However, there are challenges in restoring highly degraded ecosystems actively due to seed limitations (Ruwanza 2017) and the high-cost implications (Retief et al. 2024). Hence, restoration practitioners are often left with no choice but to implement passive restoration since it is more cost-effective. In contrast, passive restoration often does not restore fully functional ecosystems, especially where soil seed banks are depleted (Cowell 2007; Brancalion et al. 2016; Mukundamago 2016; Acosta et al. 2018; Nsikani et al. 2018; du Plessis 2021). Therefore, an active restoration strategy such as applied nucleation, that can be effective using limited resources is crucial.

Applied nucleation, which is usually considered an active restoration strategy, has been utilized as a restoration tool worldwide and proven effective in certain vegetation types, particularly in forests (Corbin & Holl 2012; Holl et al. 2020; Rojas-Botero 2020; Bechara 2021). Applied nucleation utilizes fewer resources, i.e., seeds and small nuclei to be restored (Lehman 2022). From a management perspective, applied nucleation has demonstrated greater success potential than passive restoration and highly intensive active restoration methods (Piaia et al. 2020). In the Mediterranean-type ecosystems that require effective recovery strategies, applied nucleation remains under-researched, and its potential outcomes have not been critically evaluated (Lehman et al. 2022). In the South African Swartland Shale Renosterveld (SSR) vegetation, active restoration has been practiced following applied nucleation, but with limited evaluation whether this practice can be utilized with available resources to maximise restoration outcomes. Therefore, this study made use of Tygerberg Nature Reserve (TNR) to investigate the active restoration practiced in the SSR following applied nucleation. The study aims were to (1) obtain the minimum optimal seed sowing densities and (2) determine reasons for why so many renosterveld species are difficult to restore and improve their restoration outcomes. This was achieved by assessing the ecological restoration indicators (species richness, vegetation cover, and abundance) and testing possible limiting factors (seed viability, germination, and smoke treatment) on selected species.

The findings in Chapter 2 indicated that seed sowing density had minimal influence on vegetation cover, species richness, and species abundance, except when passive restoration was used, which resulted in poor outcomes. This confirms that passive restoration is largely ineffective on highly degraded lands. Notably, restoration outcomes comparable to standard sowing densities were achieved using half the seed amount, suggesting that fewer seeds can be used to restore SSR. This highlights the feasibility of reducing sowing density and alleviating pressure on seed sources of the renosterveld remnants. The study demonstrated satisfactory cover and abundance of native species despite many species failing to establish, even though sufficient seeds were sown. Additionally, since seeds are one of the limiting resources, this implies that double the area can be restored with the same amount of seeds. Species richness remained low (12 species out of the 41 sown established), which can be partly attributed to the low seed amounts for certain species. Balancing seed mix composition – reducing seed amounts for species that establish easily and increasing the amount of seed for poorly establishing ones – could improve species richness in SSR restoration. However, understanding the reasons for the poor recruitment of these species is even more important (Chapter 3).

Dense thickets are formed over time in nuclei due to minimal spacing between established plants, indicating further seed reductions than the 50% tested here could better mimic historical vegetation (Holl et al. 2020). From a management perspective, open spaces in between established plants may be occupied by invasive species, but planting native annuals (e.g. *Dimorphotheca pluvialis* and *Leysera tenella*) early on can curb this while promoting native perennial and shrub recruitment (Hall et al. 2021). Also, excluding seed predators when restoring species may aid in mitigating seed loss, especially for those species that are prone to seed predation. Apart from the annual species, several species that were recorded establishing in this study (i.e., *Anthospermum spathulatum*, *Athanasia trifurcata*, *Eriocephalus africanus*, *Otholobium hirtum,* and *Podalyria sericea*) grow both in renosterveld and fynbos vegetation types (Rutherford et al. 2006), implying that these species are adapted to fire mechanisms. The results by Cousins et al. (2018) suggested that only certain species in SSR are fire driven, since not all species recruited under smoke treatment and in the burned sites. This study also showed little to no establishment by *Helichrysum* species (Chapter 2). Limitations such as seed viability and smoke treatments (Afolayan et al. 1997), as well as germination conditions (Byun et al. 2023), might be limiting this genus and some species which did not establish regardless of sufficient seeds.

The restoration of selected species, including *Dicerothamnus rhinocerotis* and *Helichrysum* species, in renosterveld is thus constrained by seed viability, pre-treatments, and other (unexamined) factors such as seed collection procedures, storage conditions, and other environmental variables (Chapter 3). This underscores the need for further research into their reproductive biology to support conservation efforts in threatened habitats. The findings emphasize conducting viability or germinability tests prior to restoration, as some species are sown with low seed viability – a critical factor for stored or field-sown seeds (Rao et al. 2006). Species common to both fynbos and renosterveld (e.g., *P. sericea*) exhibited fire-adapted traits, with seed dormancy broken by fire-related cues such as heat and smoke (Curtis et al. 2024). Conversely, some selected species (i.e., *Helichrysum* species) that are restricted to the renosterveld showed better seedling emergence without smoke treatment, indicating that smoke exposure might inhibit their germination (Chapter 3). Excluding these species from smoke-based pre-treatments can aid in improving their germination, since it is not fire-driven. Where restoration involves burning vegetation (e.g., Cousins et al. 2018; Curtis et al. 2024), *Helichrysum* can be actively sown post-fire. The wind-dispersal mechanism of *Helichrysum* species facilitates seed distribution, making them suitable for nucleation-based restoration approaches, which reduce the need for intensive seed collections. The study advocates for species-specific strategies in seed ecology (including seed biology) and germination pre-treatments to enhance restoration outcomes, particularly for *D. rhinocerotis* and *Helichrysum* species (Gerrits et al. 2023).

4.2 Gaps and limitations

Scaling-up ecological restoration in threatened ecosystems presents significant challenges (van Wilgen et al. 2016). These challenges are primarily attributed to resource limitations, including insufficient seed availability, the high costs associated with implementing long-term restoration projects, and the inaccessibility of other critical resources (Orrock et al. 2023; Piaia 2023). This study was limited by the inadequate seed amounts for some species, which affected species richness and abundance outcomes of the study. Additionally, seed limitation inhibited the ability to evaluate factors other than seed amounts on some species, i.e., viability and different pre-treatment techniques, which may be influencing their establishment when restoring SSR.

Seed viability is strongly influenced by storage conditions, including temperature and duration (Rao et al. 2006; De Vitis et al. 2020). Due to the absence of specialized storage facilities, seeds were predominantly stored at temperatures above recommendations, and seeds often lose viability under such conditions (Rao et al. 2006). Also, some species can be stored for a shorter duration than other species (i.e., recalcitrant species), since they cannot withstand drying out or remaining viable for a longer period. Recalcitrant species like *Albuca cooperi*, *Amaryllis belladonna*, *Hesperantha falcata*, and *Wachendorfia paniculata* should have been sown or germinated immediately after seed maturation and transplanted in the optimal restoration season. However, the space and specialised resources (i.e. nursery facilities together with plant growing resources) to practice germination of such seeds was not available. As a result, the effects of storage conditions on seed viability, particularly for species facing restoration challenges, could not be systematically investigated. These limitations highlight the need for further studies to incorporate these factors to improve restoration practices and outcomes.

Active restoration in SSR followed applied nucleation, aiming to facilitate recruitment expansion via seed dispersal from nuclei. However, recruitment was observed only within the nuclei, including for pioneer species, and little to no recruitment beyond. This limited expansion challenges the intentions of recruiting native species via natural dispersal across degraded lands. Renosterveld vegetation comprises many wind-dispersed Asteraceae species (Rutherford et al. 2006), which also dominated recruitment in this study. Since sowing began in 2019, many species have not yet matured and produced seeds. Fire-adapted species will only disperse seeds post-fire events, while some non-fire driven and fast-maturing species – such as *Dimorphotheca pluvialis*, *Lessertia frutescens* and *Leysera tenella* – also showed limited recruitment beyond nuclei. Although not formally documented in this study, which focused on within-plot sampling, these observations are based on assessments of plots four years after sowing. However, successful recruitment within nuclei occurred in areas cleared of invasive vegetation and tilled before sowing (Chapter 2), suggesting that invasive species dominance and soil conditions may hinder recruitment outside the nuclei, and that seed dispersal limitations are unlikely to be a constraint. Interventions to improve the current outcomes following applied nucleation in SSR are necessary since this practice is likely to be cost-effective and showed the potential to expand restoration scale. The TNR Restoration Plan (2018) included pre- and post-burns to control invasive grass and enhance the establishment of native species. The efficacy of fire practices on recently implemented applied nucleation remains unassessed, potentially influencing nucleation outcomes. Understanding the factors that drive the success of applied nucleation is critical for improving its ecological efficiency and cost-effectiveness (Retief et al. 2024). By identifying key contributors to positive nucleation outcomes, such as soil components, conditions of existing vegetation, fire mechanisms, viable populations within the nuclei, and dispersal mechanisms, it may be possible to optimize restoration efforts in SSR while reducing costs by targeting these specific factors. This study establishes a foundational framework to evaluate restoration success in SSR, considering the sowing density and reasons for low recruitment of some species. The findings provide a crucial baseline to guide future research on seed sowing densities and reasons for limited recruitment.

4.3 Recommendations

This study demonstrated that sowing density alone is insufficient for comprehensive SSR vegetation restoration. While the minimum sowing density tested produced outcomes similar to the standard density, species richness remained low across all treatments. Restoration practitioners are therefore advised to adopt the minimum sowing density proposed here while prioritizing strategies to enhance species richness.

To improve restoration outcomes, efforts should focus on reducing seed inputs for dominant pioneer species while increasing viable seeds of less-represented species. Alternatively, ongoing restoration efforts can build on the 12 successfully established species plus more native annual species, integrating additional species as effective strategies emerge. Species limited by seed availability should benefit from targeted seed augmentation, while shrub-trees and tree species such as *Kiggelaria africana*, *Olea europaea* subsp. *africana*, and *Searsia* spp. may require seedling planting (e.g., one per 10 m) to mitigate seed predation. Collaborative efforts can be made with indigenous plant nurseries to grow and host such trees until the optimal SSR restoration period.

Improving seed collection practices is essential. Records of collection conditions and timing should be maintained (Aronson et al., 1992; Anderson & Milberg, 1998; Mattana et al., 2010) to ensure seed quality and reference for future improvements. Seeds should be sourced from healthy mother plants to maximize viability (Anderson & Milberg, 1998). Following seed collection, proper storage conditions are also crucial; viable seeds should be stored under optimal conditions (Pedrini & Dixon, 2020). Species-specific dormancy-breaking techniques should be applied, as not all species require fire-related germination cues. Smoke treatment should be reserved for species adapted to this (Cousins et al. 2018).

Based on this study’s results, the active restoration approach (Figure 4.1) is recommended to improve current restoration practices in SSR and other renosterveld vegetation types. The approach starts with seed collection from the wild, followed by sampling seeds for pre-storage viability tests. Depending on the viability obtained, seeds are then either stored under controlled conditions (15% RH, 15°C) or recommended for research to enhance viability. In cases where advanced storage facilities are unavailable, viable seeds can be cleaned, packaged in dry, airtight containers, and refrigerated. After storage, post-storage viability tests are conducted, and seeds may undergo dormancy-breaking treatments before being sown for restoration. Germination is monitored, and further studies may be recommended to optimize conditions. Alternatively, if seeds show poor viability, vegetative propagation (cuttings) is considered, leading to direct field planting for restoration. The final step involves monitoring seedling survival in the field to assess restoration success.

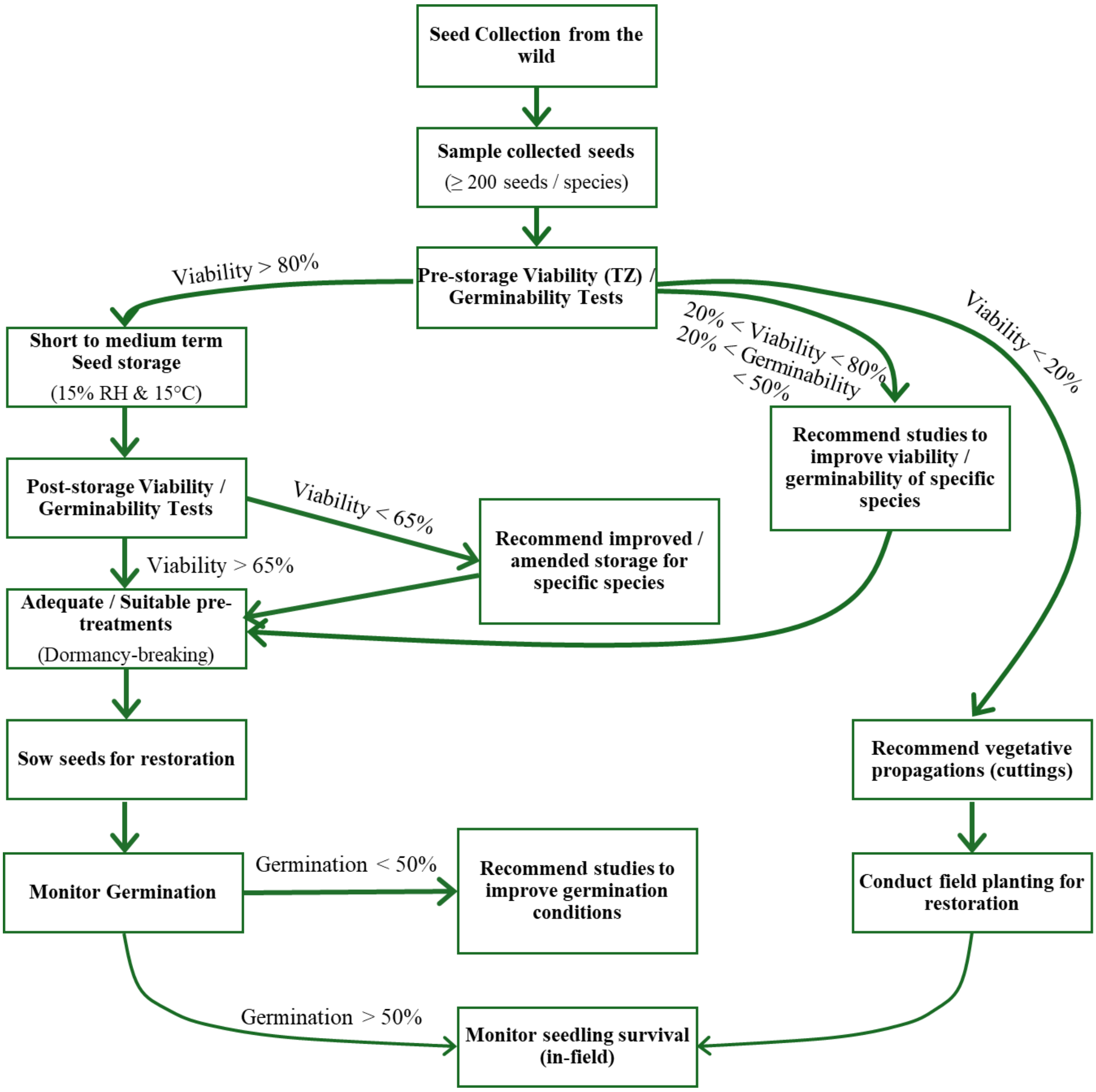

Figure 4.1: Proposed active restoration approach for Swartland Shale Renosterveld to improve restoration of species that are difficult to restore. The textbox represents the main activity to be actioned,

arrows indicate action phases/direction from one activity to another, and the text along the arrows indicates the conditions at which the action can be taken from one activity to another. Viability, germinability, and storage standards were adapted from Rao et al. (2006) and Pedrini & Dixon (2020).

References

Acosta, A. L., de Carvalho, F. A. G., Leite, M. S., Saraiva, A. M. & Metzger, J. P. W. 2018. Gaps and limitations in the use of restoration scenarios: a review. *Restoration Ecology*, 26 (6), 1108-1119. https://doi.org/10.1111/rec.12882

Anderson, L. & Milberg, P. 1998. Variation in seed dormancy among mother plants, populations, and years of seed collection. *Seed Science Research,* 8, 29–38.

Araujo, M. E. R. & Perez, D. R. 2022. From seed germination to established seedlings: a comparative evaluation in five shrub species and implications for seed-based restoration in arid lands. *Restoration Ecology*, 31 (6). https://doi.org/10.1111/rec.13862

Aronson, J., Kigel, J., Shmida, A. & Klein, J. 1992. Adaptive phenology of desert and Mediterranean populations of annual plants grown with and without water stress. *Oecologia*, 89, 17–26.

Atkinson, J. & Bonser, S. P. 2020. “Active” and “passive” ecological restoration strategies in meta-analysis. *Restoration Ecology*, 28 (5), 1032-1035. https://doi.org/10.1111/rec.13229

Bechara, F. C., Trentin, B. E., Engel, V. L., Estevan, D. A. & Ticktin, T. 2021. Performance and cost of applied nucleation *versus* high-diversity plantations for tropical forest restoration. *Forest Ecology and Management*, 491. https://doi.org/10.1016/j.foreco.2021.119088

Brancalion, P. H. S., Schweizer, D., Gaudare, U., Mangueira, J. R., Lamonato, F., Farah, F. T.... & Rodrigues, R. R. 2016. Balancing economic costs and ecological outcomes of passive and active restoration in agricultural landscapes: the case of Brazil. *Biotropica*, 48 (6), 856–867. https://doi.org/10.1111/btp.12383

Byun, C., Kettenring, K. M., Tarsa, E. E. & de Blois, S. 2023. Applying ecological principles to maximize resistance to invasion in restored plant communities. *Ecological Engineering*, 190. https://doi.org/10.1016/j.ecoleng.2023.106926

Cardoso, C. P., Bazzo, J. H. B., Marinho, J. L. & Zukareli, C. 2021. Effect of seed vigor and sowing densities on the yield and physiological potential of wheat seeds. *Journal of Seed Science*, 43 (1), 1–11. http://dx.doi.org/10.1590/2317-1545v43241586

Corbin, J. D. & Holl, K. D. 2012. Applied nucleation as a forest restoration strategy. *Forest Ecology and Management*, 265, 37–46. https://doi.org/10.1016/j.foreco.2011.10.013

Cousins, S. R., Witkowski, T. F. & Esler, K. J. 2018. Influence of fire on critically endangered swartland shale renosterveld in the cape floristic region. *Applied Vegetation Science*, 21 (1), 144–155. https://doi.org/10.1111/avsc.12348

Cowell, C. R. 2007. Protocols for fynbos restoration. *Building a sustainable future: the role of botanic gardens.* Proceedings of the 3rd Global Botanic Gardens Congress, Wuhan, China. Retrieved from: https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Protocols+for+Fynbos+restoration&btnG=#d=gs_qabs&u=%23p%3DfKh4el7tj48J

Curtis, O. E., Bond, W. J. & Chimphango, S. 2024. Diversity and fire responses in renosterveld, the forgotten relation of fynbos, in southernmost Africa. *Journal of Arid Environments*, 225. https://doi.org/10.1016/j.jaridenv.2024.105261

De Vitis, M., Hay, F. R., Dickie, J. B., Trivedi, C., Choi, J. & Fiegener, R. 2020. Seed storage: maintaining seed viability and vigor for restoration use. *Restoration ecology*, 28 (S3), S249–S255. https://doi.org/10.1111/rec.13174

Du Plessis, N. S., Rebelo, A. J., Richardson, D. M. & Esler, K. J. 2021. Guiding restoration of riparian ecosystems degraded by plant invasions: Insights from a complex social-ecological system in the global south. *AMBIO A Journal of Environment and Society*, 51, 1552–1568. https://doi.org/10.1007/s13280-021-01691-y

Gerrits, G. M., Waenink, R., Aradottir, A. L., Buisson, E., Dutoit, T., Ferreira, M. C.… & Wubs, E. R. J. 2023. Synthesis on the effectiveness of soil translocation for plant community restoration. *Journal of Applied Ecology*, 60, 714–724. https://doi.org/10.1111/1365-2664.14364

Holl, K. D., Reid, J. L., Cole, R. J., Oviedo-Brenes, F., Rosales, J. A. & Zahawi, R. A. 2020. Applied nucleation facilitates tropical forest recovery: Lessons learned from a 15-year study. *Journal of Applied Ecology*, 57, 2316–2328. https://doi.org/10.1111/1365-2664.13684

Holmes, P. M. & Richardson, D. M. 2002. Protocols for restoration based on recruitment dynamics, community structure, and ecosystem function: perspectives from South African Fynbos. *Restoration Ecology*, 7 (3), 215–230. https://doi.org/10.1046/j.1526-100X.1999.72015.x.

Holmes, P. M., Esler, K. J., Gaertner, M., Geerts, S., Hall, S. A., Nsikani, M. M.… & Ruwanza, S. 2020a. Biological Invasions and Ecological Restoration in South Africa. In: van Wilgen, B., Measey, J., Richardson, D., Wilson, J., Zengeya, T. (eds) Biological Invasions in South Africa. *Invading Nature - Springer Series in Invasion Ecology*, 14, 665–700. Springer, Cham. https://doi.org/10.1007/978-3-030-32394-3_23

Lehman, T. W. K., Esler, K. J. & Holmes, P. M. 2022. *Exploring the concept of applied nucleation as a restoration tool in a previously invaded Mediterranean climate vegetation type* (master's thesis). Stellenbosch University, Stellenbosch, South Africa.

https://scholar.sun.ac.za/server/api/core/bitstreams/7d5d784d-8dab-42ab-8300-be9484b1be3a/content

Mattana, E., Fenu, G. & Bacchetta, G. 2012. Seed production and in situ germination of *Lamyropsis microchephala* (Asteraceae) a threatened Mediterranean mountain species. *Arctic, Antarctic, and Alpine Research*, 44 (3), 343–349.

Merritt, D. J. & Dixon, K. W. 2011. Restoration seed banks—a matter of scale. *Science*, 332 (6028), 424–425. https://doi.org/10.1126/science.1203083

Moncrieff, G. R. 2021. Locating and dating land cover change events in the renosterveld, a critically endangered shrubland ecosystem. *Remote Sensing*, 13, 834. https://dx.doi.org/10.3390/rs13050834

Mukundamago, M. 2016. *Restoration of cape flats sand fynbos: the significance of pre-germination treatments and moisture regime* (master’s thesis). University of Stellenbosch, Stellenbosch, South Africa.

Nsikani, M. M., van Wilgen, B. W., Bacher, S. & Gaertner, M. 2018. Re-establishment of *Protea repens* after clearing invasive *Acacia saligna*: consequences of soil legacy effects and a native nitrophilic weedy species. *South African Journal of Botany*, 116, 103–109. https://doi.org/10.1016/j.sajb.2018.02.396

Orrock, J. L., Brudvig, L. A., Damschen, E. I., Mattingly, W. B., Cruz, J., Veldman, J. W.… & Larsen-Grey, A. L. 2023. Long-term, large-scale experiment reveals the effects of seed limitation, climate, and anthropogenic disturbance on restoration of plant communities in a biodiversity hotspot. *Proceedings of the National Academy of Sciences*, 120 (7). https://doi.org/10.1073/pnas.2201943119

Pedrini, S. & Dixon, K. W. 2020. International principles and standards for native seeds in ecological restoration. *Restoration Ecology*, 28 (S3), S286–S303. doi: 10.1111/rec.13155

Piaia, B. B., Rovedder, A., Giacomini, I. F., Felker, R. M., Stefanello, M. M. Hummel, R. & Schenato, R. B. 2022. Direct seeding for forest restoration in southern Brazil: influence of soil conditions and subtropical climate. *Revista Acta Ambiental Catarinense*, 20 (1), 01–12. https://doi.org/10.24021/raac.v20i1.6142

Rao, N. K., Hanson, J., Dulloo, M. E., Ghosh, K., Nowell, D. & Larinde, M. 2006. *Manual of seed handling in genebanks*. Handbooks for Genebanks No. 8. Bioversity International, Rome, Italy.

Retief, L., Nsikani, M. M. & Geerts, S. 2024. Using fire, planting, and topsoil translocation for restoration in South African Fynbos: assessing the success and cost-effectiveness of various restoration treatments. *Restoration Ecology*, 32 (4). https://doi.org/10.1111/rec.14102

Rojas-Boteroa, S., Solorza-Bejaranob, J., Kollmanna, J. & Teixeira, L. H. 2020. Nucleation increases understory species and functional diversity in early tropical forest restoration. *Ecological Engineering*, 158. https://doi.org/10.1016/j.ecoleng.2020.106031

Rutherford, M. C., Mucina, L. & Powrie, L. W. 2006 (Reprint 2011). Biomes and bioregions of southern Africa. In: L. Mucina, L. and Rutherford, M.C. (eds), *The vegetation of South Africa, Lesotho and Swaziland. Strelitzia 19*. South African National Biodiversity Institute, Pretoria.

Ruwanza, S. 2017. Towards an integrated ecological restoration approach for abandoned agricultural fields in renosterveld, South Africa. *South African Journal of Science*, 113, 1–4. https://doi.org/http://doi.org/10.17159/sajs.2017/a0228.

Ruwanza, S., Gaertner, M., Esler, K. J & Richardson, D. M. 2013. The Effectiveness of Active and Passive Restoration on Recovery of Indigenous Vegetation in Riparian Zones in the Western Cape, South Africa: A Preliminary Assessment. *South African Journal of Botany*, 88, 132–41. http://dx.doi.org/10.1016/j.sajb.2013.06.022.

van Wilgen, B. W., Fill, J. M., Baard, J., Cheney C., Forsyth, A. T. & Kraaij, T. 2016. Historical costs and projected future scenarios for the management of invasive alien plants in protected areas in the Cape Floristic Region. *Biological Conservation*, 200, 168–177. http://dx.doi.org/10.1016/j.biocon.2016.06.008

Supplementary material

Table S1 (Annexure 1): Previously used seed sowing densities in studies from various vegetation types of the shrublands.

Suggested seed amount (/ ha)	Vegetation type / Biome	Cleaned / Not cleaned	Individual species / mixed species / plant forms	Seed sizes	References
50 kg / ha	Renosterveld	Not cleaned	Mixed species	Mixed	Holmes, P. M. 2002. Renosterveld Restoration on Cultivated Lands at Eerste River, Western Cape: Preliminary Findings of a Pilot Study. *Unpublished report, Lafarge Quarries*.
300 kg / ha	Fynbos – Succulent Karoo transitions	Not cleaned	Mixed species	Mixed	Holmes, P. M. 2005. Results of a lucerne old-field restoration experiment at the Fynbos-Karoo interface. *South African Journal of Botany*, *71*, 326-338.
10 kg / ha	Fynbos	Cleaned	Mixed species	Mixed	Holmes, P. M., Esler, K. J., Geerts, S., Ngwenya, D. K., Rebelo, A. G., Dorse, C.… & Nsikani, M. M. 2022. *Guidelines for Restoring Lowland Sand Fynbos Ecosystems*. Self-published. ISBN: 978-0-620-98765-3.
131 kg / ha	Renosterveld	Not cleaned	Mixed species	Mixed	Mmamabolo Tshepo (pers. comm.)
0.03–3 kg / ha	Drylands	Not specified	Grasses	Small	Kilcher, M. R. & Heinrichs, D. H. 1968. Rates of seeding rambler alfalfa with dryland pasture grasses. *Journal of Range Management,* 21**,** 248–249.

Table S2: Consolidated species list for three years sown (2020 = year 3; 2021 = year 2; 2022 = year 1 after sowing) and seed densities for each year and establishment records for each year. A 'no' means there was no emergence, while a 'yes' means the species emerged satisfactorily and low means emergence was not satisfactory. For seed densities, seed amounts are represented in grams (g), unknown means there was no density data for that specific species in the specific year and species which did not have seeds at specific year are represented by '- '.

Family	***Species name***		***Sowing density (g)***			***Recruiting?***			***Conservation Status***	***Potential reason for low/no recruitment***	***Selected species***	***Reason for selecting/not selecting***
	Genus	**Species**	**2020**	**2021**	**2022**	**2020**	**2021**	**2022**				
Hyacinthaceae	*Albuca*	*cooperi*	-	-	13	-	-	No	LC	Few seeds sown, recalcitrant	-	Seeds are recalcitrant
Amaryllidaceae	*Amaryllis*	*belladonna*	-	-	29	-	-	No	LC	Few seeds sown, recalcitrant	-	Seeds are recalcitrant
Rubiaceae	*Anthospermum*	*spathulatum*	unknown	-	268	Yes	-	Yes	LC	Recruiting	-	Recruiting

Rubiaceae	*Anthospermum*	*aethiopica*	-	-	1159	-	-	No	LC	Unknown	-	N/A
Asteraceae	*Arctotis*	*calendula*	-	-	69	-	-	No	LC	Few seeds sown	-	No seeds
Iridaceae	*Aristea*	*africanus*	unknown	-	-	No	-	-	LC	No seeds	-	No seeds
Iridaceae	*Aristea*	*capensis*	-	18	-	-	No	-	LC	Few seeds sown	-	No seeds
Fabaceae	*Aspalathus*	*spinosum*	-	33	75	-	No	No	LC	Few seeds sown	-	No seeds
Fabaceae	*Aspalathus*	*cephalotes*	unknown	-	-	No	-	-	LC	No seeds	-	No seeds
Asparagaceae	*Asparagus*	*sp.*	-	-	5	-	-	No	LC	Few seeds sown	-	No seeds
Asteraceae	*Athanesia*	*trifurcata*	unknown	574	323	Low	Yes	Low	LC	Recruiting	-	Recruiting
Asteraceae	*Athrixia*	*crinata*	unknown	38	-	No	No	-	LC	Few seeds sown	-	No seeds
Asteraceae	*Athrixia*	*capensis*	-	-	34	-	-	No	LC	Few seeds sown	-	No seeds
Colchicaceae	*Baeometria*	*uniflora*	-	3	8	-	No	No	LC	Few seeds sown	-	No seeds
Asphodelaceae	*Bulbine*	*praemosa*	-	3	6	-	No	No	LC	Few seeds sown	-	No seeds
Iridaceae	*Chasmanthe*	*aethiopica*	-	-	71	-	-	No	LC	Few seeds sown	-	No seeds
Asteraceae	*Chrysocoma*	*coma-aurea*	unknown	352	1043	Low	Low	No	LC	Dormancy, viability, predation	-	N/A
Tecophilaeaceae	*Cyanella*	*hyacinthoides*	-	10	-	-	No	-	LC	Few seeds sown	-	No seeds
Asteraceae	*Dicerothamnus*	*rhinocerotis*	-	1508	4695	-	Low	No	LC	Unknown	*D. rhinocerotis*	Main species for Renosterveld
Asteraceae	*Dimorphotheca*	*pluvialis*	-	-	238	-	-	Yes	LC	Recruiting	-	Recruiting
Rutaceae	*Diosma*	*hirsuta*	unknown	-	6	No	-	No	LC	Few seeds sown	-	No seeds
Asteraceae	*Eriocephalus*	*africanus*	unknown	267	2065	Low	Low	Low	LC	Unknown	-	N/A
Asteraceae	*Euryops*	*linifolius*	unknown	279	37	Yes	Low	Yes	LC	Recruiting	-	Recruiting
Asteraceae	*Felicia*	*fruticosa*	unknown	1140	1574	No	No	No	LC	Unknown	-	N/A
Asteraceae	*Felicia*	*plumosa*	unknown	-	-	No	-	-	LC	Unknown	-	N/A
Asteraceae	*Gazania*	*krebsiana*	-	-	2	-	-	No	LC	Few seeds sown	-	N/A
Asteraceae	*Helichrysum*	*cymosum*	unknown	66	932	No	No	No	LC	pre-treatment methods	*H. cymosum*	Genus not recruiting/ No seeds
Asteraceae	*Helichrysum*	*dasyanthum*	-	-	837	-	-	No	LC	pre-treatment methods	*H. dasyanthum*	Genus not recruiting
Asteraceae	*Helichrysum*	*patulum*	unknown	488	1683	Low	Low	Low	LC	pre-treatment methods	*H. patulum*	Genus not recruiting
Asteraceae	*Helichrysum*	*teretifolium*	unknown	371	161	No	No	No	LC	pre-treatment methods	*H. teretifolium*	Genus not recruiting
Malvaceae	*Hermannia*	*althaeifolia*	unknown	-	-	No	-	-	LC	Unknown	-	N/A
Iridaceae	*Hesperantha*	*fulcata*	unknown	-	-	No	-	-	LC	Recalcitrant	-	Seeds are recalcitrant
Fabaceae	*Indigofera*	*psoraloides*	-	-	30	-	-	No	LC	Few seeds sown	-	No seeds
Achariaceae	*Kiggelaria*	*africana*	-	-	104	-	-	No	LC	Few seeds sown	-	No seeds

Fabaceae	*Lessertia*	*frutescens*	-	-	28	-	-	Low	LC	Few seeds sown	-	No seeds
Asteraceae	*Leysera*	*tenella*	unknown	92	1065	Yes	Yes	Yes	LC	Recruiting	-	Recruiting
Boraginaceae	*Lobostemon*	*argenteus*	unknown	-	-	No	-	-	LC	Unknown	-	N/A
Boraginaceae	*Lobostemon*	*capitatus*	unknown	-	-	No	-	-	VU	Unknown	-	Conservation status (VU)/ No seeds
Fabaceae	*Lotononis*	*umbellata*	unknown	4	-	No	No	-	LC	Few seeds sown	-	No seeds
Solanaceae	*Lycium*	*afrum*	unknown	-	-	No	-	-	LC	Unknown	-	N/A
Iridaceae	*Moraea*	*vegeta*	-	-	7	-	-	No	LC	Few seeds sown	-	No seeds
Asteraceae	*Nidorella*	*foetida*	-	11	-	-	No	-	LC	Few seeds sown	-	No seeds
Oleaceae	*Olea*	*europaea*	-	-	505	-	-	No	LC	Unknown	-	N/A
Hyacinthaceae	*Ornithogalum*	*thyrsoides*	unknown	-	-	No	-	-	LC	Unknown	-	N/A
Asteraceae	*Osteospermum*	*monstrosum*	-	-	32	-	-	No	LC	Few seeds sown	-	No seeds
Asteraceae	*Osteospermum*	*spinosum*	unknown	455	-	No	No	-	LC	Unknown	-	No seeds
Fabaceae	*Otholobium*	*hirtum*	-	360	2068	-	Low	Low	LC	Unknown	-	N/A
Geraniaceae	*Pelargonium*	*capitatum*	unknown	-	-	No	No	-	LC	Unknown	-	N/A
Geraniaceae	*Pelargonium*	*myrrhifolium*	unknown	-	-	No	No	-	LC	Unknown	-	N/A
Fabaceae	*Podalyria*	*sericia*	unknown	427	940	Low	Low	Low	VU	Unknown	*P. sericea*	Conservation status (VU)
Asteraceae	*Printzia*	*polifolia*	unknown	272	1311	No	No	No	LC	Unknown	-	N/A
Asteraceae	*Pteronia*	*hirsuta*	-	17	11	No	No	No	LC	Few seeds sown	-	No seeds
Iridaceae	*Romulea*	*rosea*	unknown	-	-	No	-	-	LC	Recalcitrant	-	N/A
Lamiaceae	*Salvia*	*africana-lutea*	unknown	33	-	Low	Low	-	LC	Few seeds sown	-	No seeds
Lamiaceae	*Salvia*	*aurea*	-	130	-	-	No	-	LC	Few seeds sown	-	No seeds
Anacardiaceae	*Searsia*	*glauca*	-	57	380	-	No	No	LC	Few seeds sown	-	No seeds
Anacardiaceae	*Searsia*	*laevigata*	unknown	-	-	No	-	-	LC	Unknown	-	N/A
Anacardiaceae	*Searsia*	*tomentosa*	-	58	-	-	No	-	LC	Few seeds sown	-	No seeds
Scrophulariaceae	*Selago*	*corymbosa*	-	-	74	-	-	No	LC	Few seeds sown	-	No seeds
Asteraceae	*Senecio*	*sp.*	unknown	-	-	No	-	-	LC	Unknown	-	N/A
Asteraceae	*Stoebe*	*plumosa*	-	-	2841	-	-	Low	LC	Unknown	-	N/A
Aizoaceae	*Tetragonia*	*fruticosa*	unknown	-	-	No	-	-	LC	Unknown	-	N/A
Alliaceae	*Tulbaghia*	*capensis*	-	-	16	-	-	No	LC	Unknown	-	N/A
Haemodoraceae	*Wachendorfia*	*paniculata*	-	-	-	-	-	No	LC	Few seeds sown, recalcitrant	-	Seeds are recalcitrant
Iridaceae	*Watsonia*	*borbonica*	-	-	55	-	-	No	LC	Few seeds sown	-	No seeds

Iridaceae	*Watsonia*	*marginata*	-	1	207	-	No	No	LC	Few seeds sown	-	No seeds