

**RESTORATION OF CAPE FLATS SAND FYNBOS: THE
SIGNIFICANCE OF PRE-GERMINATION TREATMENTS
AND MOISTURE REGIME.**

by

Mukundi Mukundamago

Thesis presented in partial fulfillment of the requirements of the degree of Master of Science
in Conservation Ecology, Department of Conservation Ecology and Entomology at the
University of Stellenbosch



Supervisor: Prof. K.J. Esler

Co-supervisors: Dr. M. Gaertner and Dr. P.M. Holmes

Faculty of AgriSciences

March 2016

Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Copyright © 2016 Stellenbosch University
All rights reserved

SUMMARY

The seed ecology of the Cape Flats Sand Fynbos (CFSF) vegetation's species in Blaauwberg Nature Reserve, in Western Cape South Africa, was investigated within the context of a broader restoration ecology project "Blaauwberg Ecological Restoration Project"¹.

Cape Flats Sand Fynbos (CFSF) vegetation is considered as a critically endangered vegetation type due to agricultural development, urban transformation, and degradation caused by invasive alien *Acacia* species. The City of Cape Town is clearing alien plants at Blaauwberg Nature Reserve (BBNR) in an attempt to restore this remaining CFSF fragment. These efforts are associated with challenges, since alien stands have depleted indigenous soil-stored seedbanks. The premise for this seed ecology study was that restoration of degraded CFSF is possible through applied management programmes based on the methodology and practice of active restoration. Active restoration is necessary in order to establish the major indigenous plant guilds and to revive areas that have been degraded due to the effects of the alien plants. Pre-germination treatments including smoke-derived compounds have been known to promote seed germination; however, after a burn in Blaauwberg Nature Reserve, seeds sown in the field had low germination. One reason for the low germination of re-introduced indigenous species could be a lack of pre-germination treatments prior to sowing or drought thereof.

The research on the implementation of seed ecology study was carried out in anticipation of three major outcomes. First, to determine pre-germination treatments that can improve germinability of seeds of selected indigenous target species to be used in large-scale restoration projects. Second, to assess the value of smoke as a germination cue for indigenous CFSF species; more specifically, it focuses on the germination of indigenous species following different smoke treatments. Lastly, to assess soil moisture as a potential limiting factor for seed germination and seedling establishment in lowland sand fynbos. In general, the research investigated the effect of desiccation on seed emergence and the survival of seedlings.

¹The umbrella project seeks to investigate the optimal restoration treatments and seed ecology (i.e. lack of pre-germination treatments, drought, depleted seed-banks) and a variety of challenges (i.e. drought, granivory, soil microbial ecology) that hinder the progress of restoration and the restoration potential of the reserve

Seed and soil collections were performed in Blaauwberg Nature Reserve, Western Cape, South Africa and in natural vegetation remnants along the N7 between July 2012 and January 2013. Seeds were collected, cleaned, sorted, and then stored. The main plant guilds that were selected are, Restioids, Ericoids, Proteoids, annual forbs and geophytes. Portions of seeds were set aside for different experiments (i.e. pre-germination treatments, viability test, smoke test and desiccation test). The results of the first study of viability test, germination rate, and germination period were analysed. Species showed varying responses to the different treatments with only the smoke water treatment having a consistent positive effect on seed germination for all functional groups. Only the annual, *Senecio elegans*, had a negative response to smoke water pre-germination treatment. A positive response was observed in *Thamnochortus punctatus* and *Erica plumosa* with the application of combination of smoke water and heat. Another positive response was noticed in *Babiana villosula* with the application of scarification pre-germination treatment. Additional analyses of germination period, germination lag phase and duration showed the same pattern as germinability wherein species had significant differences in germinability when there was no significant differences on germination period and vice versa.

A comparison of different viability tests (i.e. x-ray, tetrazolium and cut test) indicated that the cut test is more efficient in determining viability. Nonetheless, it is recommended to collect seeds when they are naturally dispersing since cut test is not practical in the field wherein small seeds are collected. Pre-germination treatments should be applied on target species in all restoration applications to maximise germinability and establishment. The study shows that although pre-germination treatment responses are species-specific smoke water pre-germination treatment could be used for all functional groups. Other treatments can be applied for specific species.

The second study showed that plant-derived smoke had the ability to enhance seed germination of a number of plant species. Plant-derived smoke enhancement was species-specific. Some species' germination increased significantly with pre-smoked seeds only, whereas others' germination increased significantly with pre-smoked soil only and some other species germination increased significantly with a combination of pre-smoked soil and seeds. Based on these observations, it is recommended that seeds be pre-smoked before sowing in the field, especially in the absence of a prescribed burn.

The third study focused on selected Cape Flats Sand Fynbos species that were tested for desiccation tolerance of both germinants and seedlings. I ran experiments in controlled growth chambers in parallel with desiccation tolerance investigation of seedlings of the target species in a greenhouse experiment. Results showed that almost all species germinated and they were all tolerant to desiccation.

The results within the scope of this study will help shed some light on and further enhance existing active practical restoration efforts in Cape Flats Sand Fynbos. In order for such efforts to be effective, it is important to use appropriate pre-germination treatments to enhance germination. More importantly, it should be noted that drought or desiccation are unlikely causes of poor germination/ establishment. It is probable that enhanced nutrient levels or a change in the soil microbial community might be the reason for a lack of germination/establishment in the field.

Amidst the observations made and the conclusions arrived at, it is recommended that further research should be conducted in the greenhouse alongside a field experiment to compare germination of *Acacia saligna* and indigenous CFSF species. This is because in the nursery trial, you can control some of the environmental variables such as soil nutrients and moisture and be able to investigate these factors on germination under soil conditions and the field experiment will have a variety of environmental factors involved.

OPSOMMING

Die saadekologie van die Kaapse Vlakte-sandfynbos (CFSF) plantegroei spesies in Blaauwberg Natuurreservaat, in die Wes-Kaap Suid-Afrika, is ondersoek binne die konteks van 'n breër restourasie-ekologie projek "Blaauwberg Ekologiese Restourasie Projek".²

Kaapse Vlakte-sandfynbos (CFSF) plantegroei word beskou as 'n krities bedreigde plantegroei tipe weens landbou-ontwikkeling, stedelike transformasie, en agteruitgang veroorsaak deur uitheemse *Acacia* spesies. Die Stad Kaapstad haal uitheemse plante uit in Blaauwberg Natuurreservaat (BBNR) in 'n poging om hierdie oorblywende CFSF fragment te herstel. Hierdie pogings gaan gepaard met uitdagings, aangesien uitheemse plote inheemse grond gestoorde saadbanke uitgeput het. Die uitgangspunt vir hierdie saadekologie studie was dat die herstel van gedegradeerde CFSF moontlik is deur toegepaste bestuursprogramme gebaseer op die metode en praktyk van aktiewe herstel. Aktiewe herstel is nodig om die groot inheemse plant gildes te vestig en gebiede wat ontaard het weens die gevolge van die indringerplante te laat herleef. Voor-ontkiemingsbehandelings waaronder rook-afkomstige samestellings is bekend daarvoor om ontkieming te bevorder; maar na 'n brand in Blaauwberg Natuurreservaat, het sade gesaai in die veld lae ontkieming getoon. Een rede vir die lae ontkieming van her-bekendgestelde inheemse spesies kan wees as gevolg van 'n gebrek aan voor-ontkiemingsbehandelings, voor die saai of droog daarvan.

² Die sambreel projek poog om die optimale herstel behandelings en saad ekologie (d.w.s. 'n gebrek aan voor-ontkiemingsbehandelings, droogte, uitgeputte saadbanke) en 'n verskeidenheid van uitdagings (bv. droogte, saadeterie, grond mikrobiëse ekologie) wat die vordering van herstel verhinder en die herstellpotensiaal van die reservaat te ondersoek

Die navorsing oor die implementering van die saadekologie studie is uitgevoer as gevolg van die afwagting van drie groot uitkomst. Eerstens, om voor-ontkieming behandelings te bepaal wat ontkiemingsvermoë van sade van gekose inheemse teiken spesies, wat gebruik sal word in grootskaalse herstel projekte, te kan verbeter. Tweedens, om die waarde van rook as 'n ontkiemingsteken vir inheemse CFSF spesies te evalueer; meer spesifiek, dit fokus op die ontkieming van inheemse spesies na verskillende rook behandelings. Laastens, om grondvog te evalueer as 'n potensiële beperkende faktor vir saadontkieming en saailingvestiging in laagland sand fynbos. In die algemeen het die navorsing die effek van uitdroging op saad opkoms en die oorlewing van saailinge ondersoek. Saad- en grondinsamelings is gedoen in Blaauwberg Natuurreserveaat, Weskaap, Suid-Afrika en in natuurlike plantegroei oorblyfsels langs die N7 tussen Julie 2012 en Januarie 2013. Saad is versamel, skoongemaak, gesorteer en dan gestoor. Die belangrikste plant gildes wat gekies is, is Restioids, Ericoids, Proteoids, eenjarige kruidagtige-planten en geofiete. Gedeeltes van die sade is opsy gesit vir verskillende eksperimente (d.w.s. voor-ontkiemingsbehandelings, lewensvatbaarheidstoets, rooktoets en uitdrogingstoets). Die resultate van die eerste studie van die lewensvatbaarheidstoets, ontkiemingstempo en ontkiemingstydperk is ontleed. Spesies het uiteenlopende reaksies op die verskillende behandelings getoon, met net die rookwater behandeling wat 'n volgehoue positiewe effek op ontkieming vir alle funksionele groepe gehad het. Slegs die eenjarige, *Senecio elegans*, het 'n negatiewe reaksie op rookwater voor-ontkiemingsbehandeling getoon. 'n Positiewe reaksie is waargeneem in *Thamnochortus punctatus* en *Erica plumosa* met die toepassing van 'n kombinasie van rookwater en hitte. Nog 'n positiewe reaksie is opgemerk in *Babiana villosula* met die toepassing van insnydings in die vel as voor-ontkiemingsbehandeling. Addisionele ontledings van ontkiemingstydperk, ontkiemingswagperiode en duur het dieselfde patroon as ontkiemingsvermoë getoon, waarin spesies beduidende verskille in ontkiemingsvermoë gehad en daar geen beduidende verskille in ontkiemingstydperk was en omgekeerd.

'n Vergelyking van verskillende lewensvatbaarheidstoetse (d.w.s. x-strale, tetrazolium en die snytoets) het aangedui dat die snytoets meer doeltreffend is in die bepaling van lewensvatbaarheid. Nietemin word dit aanbeveel om saad in te samel wanneer hulle natuurlik versprei, aangesien die snytoets nie prakties in die veld is nie, wanneer klein sade versamel word. Voor-ontkiemingsbehandelings moet toegepas word op teiken spesies in alle herstel toepassings om ontkiemingsvermoë en vestiging te maksimeer. Die studie toon dat hoewel voor-ontkiemingsbehandeling reaksies spesie-spesifiek is, rookwater voor-ontkiemingsbehandeling gebruik kan word vir alle funksionele groepe. Ander behandelings kan toegepas word vir spesifieke spesies.

Die tweede studie het getoon dat plant-afkomstige rook die vermoë het om die ontkieming van 'n aantal plantspesies te bevoordeel. Plant-afkomstige rook bevoordeling was spesie-spesifiek. Sommige spesies se ontkieming het aansienlik toegeneem met slegs voor-gerookte sade, terwyl ander se ontkieming aansienlik toegeneem het met slegs voor-gerookte grond en 'n paar ander spesies se ontkieming het aansienlik toegeneem met 'n kombinasie van die voor-gerookte grond en sade. Op grond van hierdie waarnemings word dit aanbeveel dat sade vooraf gerook word, voor dit in die veld gesaai word, veral in die afwesigheid van 'n voorgeskrewe brand.

Die derde studie het gefokus op uitgesoekte Kaapse Vlakte-sandfynbos spesies wat getoets is vir uitdroogtoleransie in beide spruite en saailinge. Ek het eksperimente uitgevoer in beheerde groeikaste in parallel met uitdroogtoleransie ondersoek van saailinge van die teiken spesies in 'n kweekhuis eksperiment. Resultate het getoon dat byna al die spesies ontkiem het en hulle was almal verdraagsaam teen uitdroging.

Die resultate binne die bestek van hierdie studie sal help lig werp op en verder die bestaande aktiewe praktiese herstel pogings in Kaapse Vlakte-sandfynbos verbeter. Ten einde vir sulke pogings om doeltreffend te wees, is dit belangrik om toepaslike voor-ontkiemingsbehandelings te gebruik om ontkieming te verbeter. Meer belangrik, moet daarop gelet word dat droogte of uitdroging onwaarskynlike oorsake van swak ontkieming/vestiging is. Dit is hoogs waarskynlik dat verhoogde voedingstofvlakke of 'n verandering in die grond mikrobiële gemeenskap die rede vir 'n gebrek aan ontkieming/vestiging in die veld kan wees.

Te midde van die waarnemings en die gevolgtrekkings, dink ek verdere navorsing moet gedoen word in die kweekhuis tesame met veld eksperimente, met vergelyking van waterverhoudings en voedingstofverdraagsaamheid tussen *Acacia saligna* en inheemse CFSF spesies. Dit is omrede jy in die kwekery 'n paar van die omgewingsveranderlikes kan beheer soos grondvoedingstowwe en vog en in staat is om hierdie faktore op ontkieming onder grondtoestande te ondersoek.

ACKNOWLEDGEMENTS

Funding was provided by Arcadia through the Millennium Seed Bank Programme (MSBP) of the Royal Botanic Gardens of Kew, DST-NRF Centre of Excellence for Invasion Biology (C.I.B); City of Cape Town; and South African National Biodiversity Institute (SANBI) in collaboration with Stellenbosch University (SU) (Department of Conservation and Entomology and Department of Botany and Zoology). Additional funding was provided by National Research Foundation (NRF). Khuphumla Zenze, Deon Smith and the staff members at the seed room in Kirstenbosch Botanical Gardens are acknowledged for the “Liquid smoke plus” used to pre-treat seeds. I am deeply indebted to my supervisors Dr Mirijam Gaertner, Dr Pat M. Holmes and Prof. Karen J. Esler, for their endless support, input which led to an improved thesis; and Prof. Daan Nel and Prof. Martin Kidd for assistance with statistical analysis. Erika Nortje is acknowledged for Afrikaans translation of the thesis summary.

The City of Cape Town staff, volunteers, and the MSBP staff in Kirstenbosch Botanical Gardens are acknowledged for assistance with seed collection and sorting. Zoe Poulsen and Dr John Sanni are acknowledged for proofreading the earlier drafts and final version of the thesis. Samuel Adu-Acheampong and Stuart Hall are acknowledged for assistance with smoke apparatus and experimental set-up. Suzaan Kritzinger-Klopper, Sthembiso Gumede, Vuledzani O. Mukwevho, Mashudu H. Mashau, and Mark Februarie are acknowledged for field, greenhouse, and laboratory assistance.

On a much more personal note, I would like to extend my gratitude to my family and friends who have been very supportive throughout this journey. I am highly indebted to my mom and grandmother to my babies, who has been my rock, patient with me and prayerful for my sake (nga ngoho Mulisa Wandele Uhone). I thank my sister (Dr Ahuna Mukundamago) and brothers (Nndutanyeni, Asivhashu and Avhaho Rendani Mukundamago) who were always there for me when I needed them most. Takalani Confidence Muvhango, aunty we had fun whilst transplanting the seedlings and carrying those heavy trays out of the greenhouse, let’s do it again, oh wait, I am done. Dr Natasha P. Mothapo you are a star that shines brightest enough to light my path, I am indebted. Lastly, I will like to thank my wonderful friends, who share the same Biodiversity and Ecology passion with me, Khensani Rakgalakane, Rendani Mulaudzi, Siviwe Lamani and Pfananani A. Ramulifho, guys you were always just a phone call or a cup of tea away.

DEDICATION

This thesis is dedicated to my mother Nndoweni Selinah Muvhango
Indeed “a mother's dream is to see her children grow to be happy and successful.” Mom, you
are not only a fearless woman, but a hero and my greatest inspiration. As for the thesis, this is
the beginning of mighty things yet to come.

TABLE OF CONTENTS

DECLARATION.....	I
SUMMARY.....	II
OPSOMMING.....	V
ACKNOWLEDGEMENTS.....	IX
DEDICATION.....	X
TABLE OF CONTENTS.....	XI
LIST OF FIGURES.....	XV
LIST OF TABLES.....	XVII
LIST OF APPENDICES.....	XX
CHAPTER 1: INTRODUCTION.....	1
1.1. BACKGROUND INFORMATION.....	1
1.1.1. Research Questions.....	4
1.1.2. Research Objectives.....	4
1.2. LITERATURE REVIEW.....	5
1.2.1. Invasive alien species.....	5
1.2.1.1. Introduction.....	5
1.2.1.2. What effects do <i>Acacias</i> have on fynbos?.....	6
1.2.2. Restoration.....	8
1.2.3. Seed pre-germination cues.....	9
1.2.4. Desiccation avoidance and drought tolerance.....	11
1.2.5. Research Aims.....	12
1.2.6. Thesis Outline.....	13
REFERENCES.....	15
CHAPTER 2: IMPROVING GERMINATION OF 16 CAPE FLATS SAND FYNBOS SPECIES: APPLICATIONS OF PRE-GERMINATION TREATMENTS TO INFORM LARGE-SCALE RESTORATION PROGRAMMES.....	27

ABSTRACT.....	27
2.1. INTRODUCTION.....	29
2.2. MATERIALS AND METHODS.....	31
2.2.1. Species collection, cleaning, storage and study site.....	31
2.2.2. Experimental design.....	32
2.2.2.1. Viability experiment.....	32
2.2.2.2. Germinability experiment.....	33
2.2.3. Statistical analysis.....	36
2.3. RESULTS.....	38
2.3.1. Comparison of cut test with x-ray and cut test with tetrazolium test.....	38
2.3.2. Overall germinability in response to pre-germination treatments.....	39
2.3.3. Effects of pre-germination treatments on germination rate.....	41
2.3.4. Effects of pre-germination treatments on average germination period, lag phase, duration of germination.....	42
2.4. DISCUSSION.....	43
2.4.1. Comparison of quality and viability test.....	43
2.4.2. Germination responses.....	43
2.4.3. Management implications for large-scale restoration programmes.....	47
REFERENCES.....	49
CHAPTER 3: VARIATION IN SEED GERMINATION OF CAPE FLATS SAND FYNBOS SPECIES AFTER GASEOUS SMOKE PRE-GERMINATION TREATMENT.....	66
ABSTRACT.....	66

3.1. INTRODUCTION.....	67
3.2. MATERIALS AND METHODS.....	70
3.2.1. Species selection and study site.....	70
3.2.2. Experimental Design.....	70
3.2.3. Statistical analysis.....	71
3.3. RESULTS.....	73
3.3.1. Seedling emergence in response to smoke treatment.....	73
3.3.2. Seedling emergence rate.....	74
3.4. DISCUSSION.....	75
3.4.1. Seedling emergence and rate.....	75
3.4.2. Recommendations for restoration.....	77
3.4.3. Research need.....	77
REFERENCES.....	78

CHAPTER 4:CAPE FLATS SAND FYNBOS SPECIES GERMINANTS AND SEEDLINGS’ TOLERANCE TO DESICCATION: IMPLICATIONS FOR BLAAUWBERG NATURE RESERVE LARGE-SCALE RESTORATION.....87

ABSTRACT.....87

4.1. INTRODUCTION.....	88
4.2. MATERIALS AND METHODS.....	92
4.2.1. Germination experiments.....	92
4.2.1.1. Laboratory experiments	92
4.2.1.2. Greenhouse experiments.....	94
4.2.2. Statistical analysis.....	96
4.3. RESULTS.....	96
4.3.1. Growth chamber experiment.....	96

4.3.1.1.	Pre-germination desiccation.....	96
4.3.1.2.	Post-germination desiccation.....	97
4.3.2.	Greenhouse experiment.....	98
4.4.	DISCUSSION.....	99
4.4.1	Species response to desiccation.....	99
4.4.2.	Implication for restoration.....	101
4.4.3.	Research need.....	101
	REFERENCES.....	102
	CHAPTER 5: SYNTHESIS.....	124
5.1.	SUMMARY OF FINDINGS AND RECOMMENDATIONS FOR LARGE-SCALE RESTORATION OF CFSF.....	124
5.2.	SUGGESTIONS FOR FURTHER RESEARCH.....	126
5.3.	CONCLUSIONS	127
	REFERENCES.....	128

LIST OF FIGURES

CHAPTER 2

- Figure 2.1:** Box and Whisker plot showing median numbers of full seeds of 16 CFSF target species tested for the most practical test in estimating a) seed viability using cut and tetrazolium test and, b) seed quality using cut and x-ray test. The results will be used to give restoration ecologists an idea of the suitable quality and viability test of seed during and after collection or before doing more complex seed germination.....62
- Figure 2.2:** Vector overlays on the PCA of the pre-germination treatments, showing seeds of the target CFSF species showing their relationship with the response to pre-germination treatments.....63

CHAPTER 3

- Figure 3.1:** Typical example of the randomized experimental design showing a greenhouse set-up of 4 replicates of 40 seeds each for the four pre-germination treatments including pre-germination treatments. Main trays were divided among 4 pre-germination treatments consisting of untreated seeds (US) on pre-smoked soil, smoke-treated seeds (PU) on untreated soil, pre-smoked seeds and soil (PS and control (CL). The pre-germination treatments were used to enhance seed germination on 17 target species.....83
- Figure 3.2:** Vector overlays on the Principal Component Analysis (PCA) of the smoke pre-germination treatments, showing clusters for germination response of seeds of the Cape Flats Sand Fynbos (CFSF) and their relationship with the plant-derived smoke (gaseous) pre-germination treatments. The key symbols representing the explanatory variables are shown as follows: (♦) control, (■) smoke treated-soil, (●) smoke-treated seeds and (▲) a combination of smoke-treated seeds and soil.....84

Figure 3.3: Vector overlays on the Principal Component Analysis (PCA) of the greenhouse smoke pre-germination treatments, showing clusters for germination response of seeds of the Cape Flats Sand Fynbos (CFSF) species and the variable patterns they group in terms of a) Growth form b) Regeneration mode and c) Seed size.....85

CHAPTER 4

Figure 4.1: A 20-year frequency graph showing rainfall pattern summary (April to September) for number of rain-free days after a rainfall event and/or after an initial rainfall event >50mm. Data were obtained Cape Town Weather Observation station records from 1995 to 2014.....113

Figure 4.2: Least squared means plots (mean % germination \pm SE) showing overall effects on survived germinant of a) Pre- and b) Post-germination desiccation experiment conducted in the growth chamber using CFSF species with the repeats of initial germination (pre), desiccation (dry) and rewatering maximum response in different treatments (i.e. control, 21,6,13,18 and 25 days).....114

Figure 4.3: Least squared means (mean % germination \pm SE) plot showing overall effects of seedling emergence of desiccation experiment conducted in the greenhouse using CFSF species with repeats of with the repeats of initial germination (pre), desiccation (dry) and rewatering maximum response in different treatments (i.e. control, 12, and 30 days).....115

Figure 4.4: Principal Component analysis (PCA) for regeneration mode, growth form, and seed size of seed germinants drying-down for Pre- and Post-germination treatment for 15 CFSF species showing axes of variation in Pre (initial germination), dry (desiccation), rew (rewatering), in a) 21, b) 6, c) 13, d) 18 and e) 25 days.....116

LIST OF TABLES

CHAPTER 1

Table 1.1: Names and seed characteristics of target species of Cape Flats Sand Fynbos used in the experiments (on the entire thesis) designed to give recommendations for improved germination in large-scale restoration projects. Superscript numbers used to refer to the reference source for germination cues.....26

CHAPTER 2

Table 2.1: The most practical tests in estimating quality and viability on seventeen target indigenous fynbos species tested using three test: cut, x-ray and tetrazolium test. Mean percentages \pm SD; $n=4 \times 25$, for the seed quality and viability tests. Cut and tetrazolium test results of T-test arcsine transformed percentage are compared for their variability (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS is not significant $P > 0.05$). Test with the highest quality % for each species is indicated in bold.....55

Table 2.2: List of pre-germination treatments methods based on available literature for Cape Flats Sand Fynbos species selected for use in large-scale restoration projects. X indicates treatments undertaken and Dash (–) indicates no pre-germination treatment applied.....56

Table 2.3: Effects of different pre-germination treatments on 16 target indigenous fynbos species tested under growth chamber conditions (with alternating temperatures of 21° C /10° C and a photoperiod of 10h L/14hD). Data are means of germinants percentage \pm SE $n= 4 \times 25$ seeds and results of 1-way ANOVA arcsine transformed percentage are shown (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS is not significant $P > 0.05$). Superscript letters show where the significant differences lie within species between the treatments after post hoc Fisher LSD test. Treatment with the highest germination % for each species is indicated in bold. Dash (–) indicates no data collected.....57

- Table 2.4:** The effect of different pre-germination treatment on the average seed germination period of target CFSF species. Data are mean days \pm SE $n= 4 \times 25$ seeds and results of 1-way ANOVA are shown (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS is not significant $P > 0.05$). Superscript letters show where the significant differences lie within species between the treatments after Fisher LSD and Games-Howell post hoc test. Dash (-) indicates no data collected.....58
- Table 2.5:** The effect of different pre-germination treatments and number of days on the germination lag phase of germination (expressed as days for 10% germination. Data are mean days \pm SE $n= 4 \times 25$ seeds and results of 1-way ANOVA are shown (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS is not significant $P > 0.05$). Superscript letters show where the significant differences lie within species between the treatments after Fisher LSD and Games-Howell post hoc test (species that had this test indicated by superscript number). Dash (-) indicates no data collected.....59
- Table 2.6:** Means \pm SE shows the effect of different pre-germination treatment on the germination duration (expressed as days for 90% germination) of sixteen target CFSF species. Data are means \pm SE $n= 4 \times 25$ seeds and results of 1-way ANOVA percentage are shown (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS is not significant $P > 0.05$). Superscript letters show where the significant differences lie within species between the treatments after Fisher LSD and Games-Howell post hoc test (species that had this test indicated by superscript number). Dash (-) indicates no data collected.....60
- Table 2.7:** A summary table of the study species and their recommended pre-germination treatments to be considered for restoration programmes.....61

CHAPTER 3:

Table 3.1: Effects of different smoke pre-germination treatments on percentage germination in 17 target indigenous fynbos species tested under greenhouse conditions. Data are means \pm SE, $n=4 \times 40$ seeds and results of 1-way ANOVA arcsine transformed percentage are shown (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS is not significant $P > 0.05$). Superscript letters show where the significant differences lie within species between the pre-germination treatments after Fisher LSD post hoc test. Treatment with the highest germination percentage for each species is indicated in bold.....82

CHAPTER 4

Table 4.1: Effects of different pre-germination desiccation treatments on fifteen indigenous CFSF species tested under growth chamber conditions (with alternating temperatures of 21° C/ 10° C and a photoperiod of 10h L/ 14h D). Data are untransformed means \pm SE of survived germinants (% , $n=4 \times 40$ seeds) and results of repeated measures ANOVA values are shown (* $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$). The repeats of initial germination (pre), desiccation (dry) and rewatering maximum response in different treatments (i.e. control and 21 days). Superscript letters show where the significant differences lie between the species and treatments after a Bonferroni post-hoc test, and those not significantly different are indicated by the different letters.....110

Table 4.2: Effects of different post-germination desiccation treatments on fifteen indigenous CFSF species tested under growth chamber conditions (with alternating temperatures of 21° C/ 10° C and a photoperiod of 10h L/ 14h D). Data are untransformed means \pm SE $n=4 \times 40$ seeds in percentages and results of repeated measures ANOVA values are shown (* $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$, NS is not significant $P > 0.05$). The repeats of initial germination (pre), desiccation (dry) and rewatering maximum response in different treatments (i.e. 6, 13, 18, and 25 days).....111

Table 4.3: Effects of different desiccation treatments on seventeen indigenous CFSF species tested under shaded greenhouse conditions. Data are untransformed means \pm SE $n=4 \times 40$ seeds in percentages and results of repeated measures ANOVA values are shown (* $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$). The repeats of initial germination (pre), desiccation (dry) and rewatering maximum response in different treatments (i.e. control, 12 and 30 days).....119

LIST OF APPENDICES

CHAPTER 2

Figure A.1: a-p) Cumulative curves showing percentage means of germination \pm SD, and the rate at which seeds of the target CFSF species respond to different pre-germination treatments. The x-axis is not evenly spaced at day 1 and 7, due to the fact that after experimental set-up, the first sampling was from the 7th day then continued sampling was after every 3rd day till the end of 8 weeks. The key symbols representing the treatments are shown as follows: (◆) Control, (□) Smoke water, (△) Heat, (⊕) Heat-smoke water, (○) Scarification, (▲) Scarification-smoke water.....130

CHAPTER 3

Figure B.1. a-q): Cumulative percentage germination curves depicting germination rates of the target Cape Flats Sand Fynbos (CFSF) species about application of different plant-derived smoke pre-germination treatments. The x-axis is not evenly spaced (but the actual sampling had 6 days differences in between). The key symbols representing the treatments are shown as follows: (●) Control, (■) smoke-treated seeds, (◆), combination of smoke-treated seeds and soil, (▲) smoke-treated soil.....135

CHAPTER 1: INTRODUCTION

1.1. BACKGROUND INFORMATION

The City of Cape Town (CCT) municipal area encompasses unique biodiversity, and it is located in a noticeable biodiversity hotspot known as the Cape Floristic Region (CFR). In relation to its size, the CFR is considered the richest of the 25 global biodiversity hotspots for plant diversity and endemism (Myers et al., 2000). The fynbos is a distinctive vegetation type occurring in a small belt of the Western Cape of South Africa, mainly in the winter rainfall coastal and mountainous areas associated with a Mediterranean-type climate (Rebelo et al., 2006). The fynbos environment is characterised by nutrient-poor soils, winter rains, summer drought and regular fires that burn at natural intervals of between 44 and 40 years (van Wilgen et al., 1992). Fynbos plants are characterized by being sclerophyllous (hard, tough, and leathery leaves) and microphyllous (small leaved). Physiologically species in Mediterranean environments evolved these strategies to limit the loss of water by stomatal resistance.

Fynbos occurs on well-leached, low-nutrient soils and is characterized by the presence of the following major growth components: proteoid component, which includes many species in the family of Proteaceae. They have broad isobilateral leaves with very tough cuticles to prevent water loss. They are deep rooted and can access water from far down and therefore manage to carry on growing even when it is dry. This is one reason why they form the dominant overstorey in fynbos (Cowling et al., 1997). Most proteoids have a serotinous cone and can only release seeds after fire (Heelemann et al., 2008) and have resprouters that are less reliant on serotinous cones (Le Maître and Midgley, 1992).

The Restioid component belongs to the Restionaceae or the Cape Reed Family. The reed-like Restionaceae replaces grasses on nutrient-poor soils in Mediterranean-type climate. Restioids have reduced or absent leaves and tough wiry stems (Campbell, 1985) to avoid loss of water; their seeds are often dispersed by Ants,

The ericoid or heath component consists of various families, for instance, Ericaceae, Asteraceae, Rutaceae, and Rhamnaceae. The upper surface of the ericoids' leaves is waxy and the sides are curled under until only a small slit of hairy lower surface is showing that is a characteristic to avoid leaf water content loss (Branch, 1988). Ericoid species with shallow roots are able to make efficient use of moisture in the surface layers of soil when it is present and even after small amounts of rain (Cowling and Richardson, 1995). Ericoids store their seeds in the soil, which consists of both reseeders and resprouter species.

Geophyte Geophytes, as opposed to Ericoids, has component, which includes families such as, Amaryllidaceae, Iridaceae, Liliaceae, and others. The fynbos environment is rich in geophytes. Some species die down completely during summer and only grow leaves during winter (Holmes and Richardson, 1999). The geophyte species are perennial plants with underground storage organs such as bulbs, rhizomes, and tubers that propagate through buds below the surface (Esler et al., 1999; Keeley et al., 2012). In total, the CFR supports over 8500 plant species, of which 70% are endemic (Myers et al., 2000). The CCT has a distinctive set of eleven vegetation types of which the Cape Flats Sand Fynbos (CFSF) is critically endangered (Driver et al., 2012).

Cape Flats Sand Fynbos is endemic to the City of Cape Town. This study site was the Blaauwberg Nature Reserve (BBNR) located about 25 km north from the city centre of Cape Town, on the West Coast, between Big Bay, Bloubergstrand, and Melkbosstrand in the Western Cape, South Africa. Since BBNR is an important part of the CCT's Biodiversity Network, it was proclaimed a nature reserve in 2007 (Holmes et al., 2008). The BBNR conserves a unique combination of three vegetation types, of which CFSF is the main vegetation type. The three threatened vegetation types are namely, Cape Flats Dune Strandveld (endangered), Swartland Shale Renosterveld (critically endangered), and Cape Flats Sand Fynbos (critically endangered) (Rebelo et al., 2006), as well as the transition zones between them. Within these vegetation types in the reserve, 559 plant species have been identified, 47 of which are listed in the Red List of threatened plant species (Rebelo et al., 2006; Raimondo et al., 2009; Driver et al., 2012). The Cape Flats Sand Fynbos vegetation is a national conservation priority because of its critically endangered status. Only 14% of the CFSF remains, which is below the 30% national conservation target (Rouget et al., 2004).

Furthermore, half of the remaining indigenous vegetation is dilapidated (Holmes et al., 2008). The main threat to this vegetation type is habitat destruction caused by cultivation and urban sprawl, with invasive alien species (i.e. *Pennisetum clandestinum* Hochst. ex Chiov., *Acacia saligna* (Labill.) Wendl., *Pinus radiata* D. Don., *Acacia cyclops* A. Cunn. ex G. Don, *Eucalyptus salmonophloia* F.Muell.) being the second largest threat (Rebelo, 1996; Rebelo et al., 2005). Australian *Acacias* form a large proportion of the invasive species found in the vegetation type. One can therefore, infer that Australian *Acacias* are one of the biggest concerns confronting managers of vegetation. *Acacia saligna*, as the most dominant invasive species in the BBNR was introduced to South Africa in 1845 for dune stabilisation, and has since invaded large areas in the lowlands (Shaughnessy, 1980; Mehta, 2000; Poynton, 2009). Invasive *Acacia* species are known to change fire regimes (i.e., fire frequency and intensity) and alter nutrient cycling leading to irreversible changes in the indigenous ecosystem (Richardson et al., 2000, Le Maître et al. 2011). Restoration of this degraded vegetation is thus a priority.

Large-scale control of invasive *Acacia* species in the Cape lowlands has been a priority for several years (Holmes et al., 2000; Holmes, 2008). However, indigenous species recovery following *Acacia* removal is often poor (Cowell, 2013; Stuart Hall, Unpublished data). Previous studies have shown that the recruitment of indigenous seedlings is reduced by the depletion of indigenous seed banks and competition from the dense *Acacia* seedling recruitment (Holmes, 2002). This implies that control operations should include re-introduction of indigenous species (active restoration). Active restoration could involve direct sowing of seeds after an area is cleared of invasive species (Gaertner et al., 2011; Ruwanza et al., 2013).

1.1.1. Research Questions

At the BBNR, alien clearing operations have been followed by different active restoration interventions such as sowing of indigenous species. However, to date, germination success has been very poor (Stuart Hall, Unpublished data). Poor germination of sown seeds in the field may have been due to pre-germination factors, such as seed granivory and/or post-germination factors, or drought following germination and herbivory. Another reason for poor germination could be due to the lack of appropriate pre-germination cues that overcome dormancy. This project is therefore designed to gain better understanding about the limitations of germination and seedling recruitment in seeds sown for active restoration projects. The first part of my study focuses on pre-germination factors (i.e., testing different pre-germination cues) whereas the second part focuses on post germination factors (i.e., drought as a reason for poor seedling establishment).

1.1.2. Research Objectives

It is known that germination of fynbos species is triggered by heat-shock and smoke cues (De Lange and Boucher, 1990; Brown, 1993; Keeley and Bond, 1997). Studies on germination cues (e.g. heat, smoke, scarification etc.) have been performed on other fynbos species from mountain fynbos ecosystems (Brown, 1993; Brown et al., 1994; Brown et al., 2004), but it is not the focus of this study. The aim of my first study is to determine pre-germination treatments that may improve germinability of the selected indigenous seeds of target species to be used in the large-scale restoration project.

After large-scale clearing of invasive *Acacias*, BBNR was burned and indigenous species were re-introduced a month later by sowing seed mixes of main guilds, but germination success was low: one reason could be that seeds were not pre-treated with smoke. Smoke treatment or the lack of it thereof could be the reason for low germination success (Keeley and Fotheringham, 1998; van Staden et al., 2000; Ooi, 2007). The aim of my second study/experiment was to test different smoke treatments of soil only versus smoke treatment of seed only versus a combination of smoked soil and seed), on a variety of CFSF species representing different guilds. Studies were conducted in the greenhouse under controlled conditions.

As already highlighted, the lack of soil moisture might be responsible for reduced seed germination and seedling establishment as the seedling stage is the most vulnerable in a plant's life history, when it is most prone to drought (Esler and Philips, 1992; Mustart et al., 2012), competition, herbivory and disease (Silvertown et al., 2012). To date, seed drying down studies have been limited to Proteaceae and some other non-target species (Mustart et al., 2012). The third aim of this study was hence to investigate soil moisture as a potential limiting factor required for seed germination and seedling establishment on a variety of CFSF species. In order to examine the impact of drought/desiccation on CFSF species germination I conducted a growth chamber and greenhouse experiments.

1.2. Literature Review

Approximately 50% of the CFSF vegetation has been transformed through urban development and what remains is disturbed by agriculture and past deliberate planting of invasive species, most notably Australian *Acacias* to stabilize the mobile dunes. In this unique diversity of the CFSF vegetation, invasions by alien plants are a major concern.

1.2.1. Invasive alien species

1.2.1.1. Introduction

Invasive alien species are the second largest threat to the Cape Flats Sand Fynbos (Low and Rebelo, 1995), and are recognised as the most common form of disturbance in persisting vegetation remnants. Invasive aliens are species that have been introduced into areas outside of where they naturally occur, either intentionally or accidentally, by humans (Richardson et al. 2000). Invasive alien species can have significant ecological and economic impacts (Lockwood et al., 2007) and are a threat to biodiversity. Invasive alien species can also have major effects on resource availability, hence reducing the relative abundance of indigenous species (Low and Rebelo, 1995; Holmes et al., 2000). These invasive these invasive species often out-compete indigenous fynbos plants by depriving them of available resources such as light, water and nutrients (MacDougall and Turkington, 2005; van Wilgen et al., 2001). At the same time, they grow faster following fire and can therefore out-grow indigenous species that take a longer period to reach reproductive age (Mehta, 2000).

1.2.1.2. What effects do *Acacias* have on fynbos?

Invasive alien species within the CFR compete aggressively with the indigenous vegetation during post-fire periods, which results in mono-specific stands of alien species (Higgins et al., 2001). The dominant invasive alien species in the CFR are pines, hakeas, and *Acacias* (Shaughnessy, 1980). *Acacias* are the largest invasive alien genus in the CFR and are from the legume family (Fabaceae, Mimosoideae). Most *Acacias* are successful invaders as they are able to adapt to recurring fires at intervals of between 5 and 40 years and are capable of growing in nutrient-deficient soils since they are nitrogen-fixers, thus altering nutrient cycling patterns (Witkowski, 1991; Yelenik et al., 2004). The problematic species noticed in in this study is the *Acacia saligna*, which grows in fynbos, near streams and wetlands and on the coastal plain where there is a high water table.

Port Jackson willow (*Acacia saligna* (Labill.) H. Wendl.) is an easily adapting and fast-growing tree indigenous to south-Western Australia (Midgley and Turnbull, 2003). It occurs as a shrub form, which is usually 2-5m tall and as a small tree form which can grow as high as 5-10m, with a short but definite main stem (Henderson, 2001). It has an average life-span of 30-40 years. It is used for soil stabilization, tannin production, fuel wood, windbreaks, animal fodder, and ornamental use in many countries including South Africa in the CFR (Midgley and Turnbull, 2003). However, it might be of value in some areas, *Acacia saligna* is an invasive alien species with several negative impacts (van Wilgen and Richardson, 1985).

Acacia saligna enhances soil nutrient levels through Nitrogen (N₂) fixing bacteria inside its roots nodules, which gives the species a competitive advantage over indigenous species (Mehta, 2000). In some cases, *Acacia* invasions can lead to change in nitrogen cycling, which can promote its own re-invasion or can lead to secondary invasions of other weedy species after clearing and may prevent the re-establishment of indigenous species (Yelenik et al., 2004; Holmes, 2008; Gaertner *et al.*, 2011). Persistence of soil-nutrient changes caused by invasive species after their removal has been observed in several studies (Kelly, 1998; Ehrenfeld, 2003; Yelenik, 2004; Marchante et al., 2009).

Acacia saligna can efficiently use available soil resources at rates exceeding that of indigenous plants, thus giving it a competitive advantage over indigenous plants (Holmes and Cowling, 1997). *Acacia saligna* spreads rapidly and forms dense, often impenetrable vegetation that tends to deprive indigenous plants of available growth resources (notably water, light, and oxygen) (Witkowski, 1991; Musil, 1993; Cowling and Richardson, 1995; Lockwood et al., 2007). Furthermore, *Acacia saligna* tends to reduce soil water availability, since it forms large canopies accompanied with high rates of transpiration (Musil, 1993), leaving no surplus for indigenous plants.

Acacia saligna has large soil-stored seed banks with high levels of seed viability and a strong re-sprouting capability (Holmes and Cowling, 1997; Strydom et al., 2012). Seeds stay dormant in soil for up to 50 years. According to Holmes (1989), 45% of the seeds decay in the first year, but this rate diminishes at a near log-linear pace over time. This persistence may hamper the restoration of indigenous communities, since fynbos seedlings are outcompeted at the establishment phase. The indigenous soil seed bank is often depleted following alien invasion because seeds germinate unsuccessfully, decay or are buried to a deeper soil profile (Holmes and Newton, 2004). Research has shown that after three generations of invasion (i.e., three fire cycles); indigenous seed banks are lost (Le Maître et al., 2011). Most of the dominant overstorey species are serotinous (*Leucadendron* and *Protea* species) which have short seed life spans; however, these overstorey species comprise relatively few species in the community (Le Maître and Midgley, 1992). Such species are the first to disappear following invasion. Species with short-lived seed banks are lost earlier than those with long-lived seed banks.

A. saligna is difficult to manage. Attempts at controlling the species are hampered by its ability to resprout from its roots and get its large persistent seed banks. Reducing the seed bank of *A. saligna* by means of biological control (van Wilgen et al., 2000; Paynter and Flanagan, 2004) in combination with mechanical and chemical control methods is seen as an important integrated control approach (Holmes, 1990). However, the management of this invasive alien will also require active restoration of the indigenous vegetation to intensify recovery and to limit *A. saligna*'s reinvasion.

1.2.2. Restoration

Restoring Cape Flats Sand Fynbos after invasive alien species clearance is critical to strive towards the national conservation target (Rouget et al., 2004). Restoration also has the potential to ensure ecosystem stability wherein indigenous plant growth provides adequate plant cover and community structure (shrubs, herbs, forbs, grasses, etc.), and good indigenous species guild representation (Holmes and Cowling, 1997; Gaertner et al., 2011). Although mechanical clearing of dense stands of *Acacias* is very costly, it is in largely the only practical way to remove these stands (Cowling and Richardson, 1995). Clearing can precede burning this is generally complemented with follow-up clearing events since there is often a flush of seedlings stimulated by fire as *Acacia* seeds germinate *en masse* after fire (Van Wilgen and Richardson, 1985).

Seeds are the cheapest way to re-introduce indigenous species by orders of magnitude (Holmes, 2002), but the lack of naturally available seeds can be a major limiting factor in restoration success. Active restoration can include broadcast sowing of seed and/or directly planting seeds, plugs or seedlings (Cowell, 2013). Several factors can hinder the post-fire establishment of the seedlings (Florentine, 2008). These include unfavourable soil conditions (Smith et al., 1992; Cowell, 2013), competition from established plants, herbivory (Le Maître et al., 2011), competition from invasive alien species (Mehta, 2000), dormancy (Baskin and Baskin, 2004) and climatic conditions (Midgley et al., 2005). However, the use of seed comes with dormancy challenges that require the knowledge of pre-germination requirements necessary to overcome and enhance germination.

1.2.3. Seed pre-germination cues

Fire in the fynbos is a crucial trigger that initiates recruitment and succession. In the fynbos, chaparral, Kwongan and other Mediterranean-climate communities, fire influences seed germination. Germination triggers of plant species tend to mirror the favourable conditions for their emergence and establishment (Fenner, 1987). Fire-prone ecosystems tend to provide conditions favourable for seed germination of many species after the passage of fire. This is so because fire improves resource availability (Christensen and Muller, 1975; Bond and van Wilgen, 1996; Duckworth et al., 2000). The window for germination after fire is limited. Hence, one needs to understand why plants produce seeds with dormancy that can be overcome by direct and indirect fire-related germination cues (Bond and van Wilgen, 1996).

Seed has different types of dormancy (i.e. physical, physiological, morphological or morphophysiological) (Baskin and Baskin, 2004). A dormant seed is one that does not have the capacity to germinate under combinations of normal environmental factors that are otherwise favourable for its germination (Baskin and Baskin, 2004). Since many seeds are dormant, it is important to understand how to overcome dormancy and facilitate germination (Baskin and Baskin, 2004; Ooi, 2007). When seeds are used for restoration activities, it is therefore important to pre-treat seeds to overcome dormancy using suitable germination cues or pre-germination treatments.

Fire characteristics (i.e. germination cues) have a function in releasing dormancy and or stimulating germination (Bond and van Wilgen, 1996). Germination cues include high temperatures (Keeley, 1987; Trabaud and Oustric, 1989), alternating temperatures (Brown and Botha, 2004), cold stratification (Ooi, 2007) and heat (Jeffrey et al., 1988; Musil and de Witt, 1991; Keeley and Fotheringham, 1997),), which help to break the seed coat so that water or moisture can enter to allow germination. The exposure of the soil after a burn in combination with the heat and smoke stimulates the germination of exposed and buried seeds (van Wilgen et al., 1992; Brown and Botha, 2004; Fisher et al., 2009). However, the success of seed germination also will depend on an appropriate season of burn (Auld and O'Connell, 1991; Bond and van Wilgen, 1996).

Plant-derived smoke is known to improve seed germination (van de Venter and Esterhuizen, 1988; Brown, 1993; Dixon et al., 1995; Keeley and Bond, 1997; Keeley and Fotheringham, 1998; Light et al., 2010). Since chemicals in smoke-derived extracts i.e. butenolides, ethylene, and ammonia can stimulate seed germination (Brown, 1993; van Staden et al., 2000; Flematti et al., 2004; Light et al., 2010). According to Ooi (2007), smoke had a positive effect on seeds that remained morphologically dormant, after physiological dormancy had been broken during burial or stratification, and seed germination increased significantly after pre-treatment with smoke. He further states that smoke increases the sensitivity of seeds to the hormones that promote embryo growth and therefore overcome morphological dormancy (Van Staden et al., 2000), which could increase the proportion of seeds within a seed lot that germinate.

Scarification (Cowell, 2013), desiccation (Mustart et al., 2012) and a combination thereof also tend to improve seed germination (De Lange and Boucher, 1990; Brown and Botha, 2004). These findings have led to the interpretation that seed germination of many Mediterranean-type plant species is convergent, linked to evolution under fire (Keeley and Bond, 1997); indeed, many species respond positively to fire-related germination cues. To improve restoration outcomes, the study sought to determine if gaseous smoke enhances seed germination of CFSF vegetation and if the absorption of active compounds should occur in both soil and seeds or seed alone.

The establishment of indigenous fynbos individuals after alien clearing at the Cape Flats Sand Fynbos vegetation is low compared to the high levels of recruitment by *Acacia saligna* seeds (Stuart Hall, unpublished). Knowledge of the germination ecology of these species is therefore required to shed some light on the pre-germination treatments that may improve their germination prior to sowing. Findings have been published on propagation of CFR plant species with the application of suitable pre-germination treatments to improve their germination responses (Jeffrey et al., 1988; De Lange and Boucher, 1990; Musil and de Witt, 1991; Brown et al., 1998; Brown and Botha, 2004). However, very little is known about lowland Fynbos species germination, and virtually nothing about Cape Flats Sand Fynbos species.

1.2.4. Desiccation avoidance and drought tolerance

Soil moisture is a limiting factor for seed germination and seedling survival. Plants have developed two different strategies to cope with low soil moisture levels, desiccation tolerance or desiccation avoidance. Desiccation tolerance is defined as the ability of a living structure to survive drying with low (<50%) relative humidity and maintain low intracellular water concentrations. Drought avoidance is survival of low environmental water availability while maintaining high internal water contents (Alpert, 2005). Desiccation avoidance and tolerance contribute to a plant's drought tolerance in different ways and in varying proportions (Turner, 1986). The species that exhibit a desiccation avoidance response have the ability to survive periods of drought by maintaining relatively high water potentials. In contrast, species decreasing their water potential during drought exhibit a desiccation tolerance response. The plant species ability to avoid or tolerate desiccation depends on their response to water stress via stomatal conductance (Turner, 1986). This includes a range of anatomical, morphological, and physiological features that allow water usage patterns. The features for drought resistance include the leaf life-span, rooting depth, sclerophylly, and leaf size. Plants have different strategies in response to the lack of water (Levitt, 1972); these are the drought tolerance (physiological strategy) and drought avoidance (by a deep root system) or by having at least part of the life cycle as dormant seeds (temporal avoidance of drought).

In comparison with other Mediterranean-type ecosystems, Fynbos is nutrient-poor and has less water stress (Stock et al., 1992), which means that water generally is not a limiting factor in the Fynbos. Invasion of Fynbos ecosystems by Australian *Acacias* and climate change are a major concern about drought/desiccation. This is of great importance, especially given that future climate change predictions for the Western Cape show the probability of hotter and drier conditions (Midgley et al., 2002). Actually, the expected declines in rainfall may drive some indigenous plant species further than their drought thresholds, causing extensive mortality of more vulnerable species (Midgley et al., 2005).

In invaded ecosystems, water depletion is considered one of the most significant impacts of Australian *Acacia* species (Le Maître, 2004). The increased water use is likely a result of larger above-ground biomasses of Australian *Acacia* stands compared to indigenous vegetation. Australian *Acacia* seedlings tend to develop roots 1.5 to 4-fold longer than co-occurring indigenous species, which penetrate deeper into the soil profile (Witkowski, 1991; Morris et al., 2011). This usually occurs at a faster rate than indigenous vegetation with no reduction in above-ground biomass (Musil, 1993). This rapid root growth of *Acacia* seedlings gives them competitive ability for water, especially during drought periods, giving them advantage over indigenous species. Another aspect that gives *Acacias* a better drought tolerance trait is the phyllodes because of their sclerophyllous nature (Pasquet-Kok et al., 2010). Hence, a difference in water usage compared to the Fynbos growth forms. In dry, sand plain environments, desiccation tolerance likely is an important survival mechanism that ensures post-fire establishment from seed (Groom, 2002; Mustart et al., 2012).

Species indigenous to fire-prone Fynbos vegetation in this Mediterranean-climate region tend to have a distinct trigger for seed germination largely after fire, which occurs during the favourable, moist winter period (Bond, 1984; Brits, 1986; Mustart and Cowling, 1993). Besides lack of pre-germination treatments, there is also a knowledge gap regarding the causes of low germination of indigenous Fynbos plants after alien clearing. Studies on the effects of drought on Mediterranean plant species have shown that some species' seedlings are more tolerant of drought than others (Coops and van der Velde, 1995; Groom, 2002; Mustart et al., 2012); this study will focus on selected seeds of CFSF species looking at different levels of moisture regimes.

1.2.5. Research Aims

1. The first study (Chapter 2) aimed to determine pre-germination treatments that can improve germinability of the seeds of target CFSF species to be used in large-scale restoration projects.

2. The second aim of the research was to assess the value of smoke as a germination cue for indigenous CFSF species (Chapter 3). More specifically, it focused on the germination of indigenous species following different smoke treatments. The study tested smoke treatment of soil only versus smoke treatment of seed only versus a combination of smoked soil and seed. Germination and seedling growth experiments were carried out in a greenhouse. Germination and growth data were used to determine whether there are links between smoke treatment (soil and/or seeds) and seed germination and establishment. Findings of this study can be used to recommend germination cues in active restoration of CFSF after alien clearing.

3. The third aim of the research was to assess soil moisture as a potential limiting factor for seed germination and seedling establishment in lowland sand Fynbos (Chapter 4). More specifically the research investigated the effect of drought on seed germination and seedling survival. Two experiments were performed, one in a greenhouse and the other in the growth chamber. The data obtained from the drying-down experiment were used to assess the influence of moisture on seed germination and seedling establishment. Findings of this study can be used to improve active restoration of Cape Flats Sand Fynbos after removal of invasive Australian *Acacia* species.

1.2.7. Thesis Outline

This thesis explores the seed ecology of a selection of indigenous species of the Cape Flats Sand Fynbos vegetation (Table 1.1) in Blaauwberg Nature Reserve, with the aim of providing insight into the use of different pre-germination treatments giving specific recommendations for smoke treatments for large-scale restoration. It is further investigating drought as a potential limiting factor for Fynbos species germination following removal of Australian *Acacia* species. This thesis has been written as a series of manuscripts prepared for publication in the South African Journal of Botany; each chapter is therefore written as a stand-alone paper. As such, their introductions are detailed, quoting relevant literature for the ecological principles being investigated and setting the scene for each of the aims addressed. However, there are some repetitions in the introductions (chapter one) because of this approach.

Chapter 2 is an experiment investigating seed viability to determine germination success and the use of pre-germination treatments to enhance germination of 16 Cape Flats Sand Fynbos species (Aim 1). Chapter 3 describes an experiment aimed at assessing an effective way of enhancing seed germination using gaseous smoke pre-germination treatment of the (Aim 2). Chapter 4 reports on germinants and seedling survival from different moisture regimes in both greenhouse and growth chamber experiments conducted on 17 species (Aim 3). Chapter 5 final chapter outlines the main findings of chapters two to four and includes recommendations for management.

REFERENCES

- Alpert, P., 2005. The limits and frontiers of desiccation-tolerant life. *Integrative and Comparative Biology* 45, 685-695.
- Auld, T.D., O'Connell, M.A., 1991. Predicting patterns of post-fire germination in 35 eastern Australian Fabaceae. *Australian Journal of Ecology* 16, 53-70.
- Baskin, C.C., Baskin, J.M., 2004. Germinating seeds of wildflowers, an ecological perspective. *Hortechonology* 14(4), 467-473.
- Bond, W.J., 1984. Fire survival of Cape Proteaceae - influence of fire season and seed predators. *Vegetatio* 56, 65-74.
- Bond, W.J., van Wilgen, B.W., 1996. *Fire and plants: population and community biology*, series 14. Chapman and Hall, London.
- Branch, G.M., 1988. *Explore the Cape flora and its animals*. Struik publishers, Cape Town.
- Brits, G.J., 1986. The effect of hydrogen peroxide treatment on germination in Proteaceae species with serotinous and nut-like achenes. *South African Journal of Botany*, 52, 291-293.
- Brown, N.A.C., 1993. Promotion of Fynbos seeds by plant-derived smoke. *New Phytologist* 123, 575-583.
- 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, 559-581.
- Brown, N.A.C., Botha, P.A., Johnson, T., Prosch, D.S., 1998. Propagation of Cape Wildflowers from seed. *Proceedings of the 3rd International Botanical gardens Conservation Congress, Kirstenbosch, Cape Town.* pp. 1-10. < http://www.bgci.org/congress/congress_1998_cape/html/brown.htm >

- Brown, N.A.C., Jamieson, H., Botha, P.A., 1994. Stimulation of germination in South African species of Restionaceae by plant-derived smoke. *Plant Growth Regulation* 15, 93-100.
- Campbell, B.M., 1985. A classification of the mountain vegetation of the Fynbos biome. *Memoirs of the Botanical Research Institute*, Department of Agriculture and Water Supply, Republic of South Africa, Pretoria.
- Christensen, N.L., Muller, C.H., 1975. Effects of fire on factors controlling plant growth in *Adenostoma* chaparral. *Ecological Monographs* 45, 29-55.
- Coops, H., van der Velde, G., 1995. Seed dispersal, germination and seedling growth of six helophyte species in relation to water-level zonation. *Freshwater Biology* 34, 13-20.
- Cowell, C., 2013. Investigating the most favourable seed establishment methods of restoring sand Fynbos on old fields. Unpublished Master of Technology thesis, Cape Peninsula University of Technology.
- Cowling, R.M., 1992. The ecology of Fynbos: nutrients, fire and diversity, pp. xi-411.
- Cowling, R.M., Richardson, D.M., 1995. Fynbos: South Africa's unique floral kingdom. Tien Wah press, Singapore.
- Cowling, R.M., Richardson, D.M., Mustart, P.J., 1997. Fynbos. In: Cowling, R.M., Richardson, D.M., Pierce, S.M., (Eds.). *Vegetation of Southern Africa*. Cambridge University Press, New York. pp. 99-123.
- De Lange, J.H., Boucher, C., 1990. Autecological studies on *Audouinia capitata* (Bruniaceae). 1. Plant derived smoke as a seed germination cue. *South African Journal of Botany* 55, 700-703.
- Dixon, K.W., Roche, S., Pate, J.S., 1995. The promotive effect of smoke derived from burnt native vegetation on seed germination of western Australian plants. *Oecologia* 101, 185-192.

- Driver A., Sink, K.J., Nel, J.N., Holness, S., van Niekerk, L., Daniels, F., Jonas, Z., Majiedt, P.A., Harris, L., Maze, K., 2012. *National Biodiversity Assessment 2011: An assessment of South Africa's biodiversity and ecosystems. Synthesis Report*. South African National Biodiversity Institute and Department of Environmental Affairs, Pretoria.
- Duckworth, J.C., Kent, M. and Ramsay, P.M. 2000. Plant functional types: an alternative to taxonomic plant community description in biogeography? *Progress in physical geography* 24(4):515-542.
- Ehrenfeld, J.G., 2003, Effects of exotic plant invasions on soil nutrient cycling processes. *Ecosystems* 6, 503–523.
- Esler, K.J., Phillips, N., 1994. Experimental effects of water-stress on semiarid karoo seedlings: implications for field seedling survivorship. *Journal of Arid Environments* 26, 325–337.
- Esler, K.J., Rundel, P.W., Vorster, P., 1999. Biogeography of prostrate-leaved geophytes in semi-arid South Africa: hypotheses on functionality. *Plant Ecology* 142, 105-120.
- Fenner, M., 1987. *Seed Ecology*. Chapman and Hall, London.
- Fisher, J.L., Loneragan, W.A., Dixon, K., Veneklaas, E.J., 2009. Soil seed bank compositional change constrains biodiversity in an invaded species-rich woodland. *Biological Conservation* 142 (2), 256-269.
- Flematti, G.R., Ghisalberti, E.L., Dixon, K.W., Trengove, R.D., 2004. A compound from smoke that promotes seed germination. *Science* 305, 977.
- Florentine, S.K., Graz, F.P., Ambrose, G., O'Brien, L., 2011. The current status of different age, direct-seeded revegetation sites in an agricultural landscape in the Burrumbeet region, Victoria, Australia. *Land Degradation and Development* 1-9.

- Gaertner, M., Richardson, D.M., Privett, S.D.J., 2011. Effects of alien plants on the ecosystem structure and functioning and implications for restoration: insights from three degraded sites in South African fynbos. *Environmental Management* 48(1), 57-69.
- Groom, P.K., 2002. Seedling water stress response of two sandplain *Banksia* species differing in ability to tolerate drought. *Journal of Mediterranean Ecology* 3(4), 3-9.
- Heelemann, S., Proches, S., Rebelo, A.G., van Wilgen, B.W., Porembski, S., Cowling, R.M., 2008. Fire season effects on the recruitment of non-sprouting serotinous Proteaceae in eastern (bimodal rainfall) fynbos biome, South Africa. *Austral Ecology* 33, 119-127.
- Henderson, L., 2001. Guide to the identification of the species. In: Alien weeds and invasive plants: A complete guide to declared weeds and invaders in South Africa. Agricultural Research Council. Pretoria. Pp. 8-227.
- Higgins, S.I., Richardson, D.M., Cowling, R.M., 2001. Validation of a spatial simulation model of a spreading alien plant population. *Journal of Applied Ecology* 38, 571-584.
- Holmes, P., 2002. Depth distribution and composition of seed banks in alien-invaded and uninvaded fynbos vegetation. *Austral Ecology* 27, 110-120.
- Holmes, P., 2008. Optimal ground preparation treatments for restoring lowland fynbos vegetation on old fields, *South African Journal of Botany* 74, 33-40.
- Holmes, P., Cowling, R.M., 1997. The effects of invasion by *Acacia saligna* on the guild structure and regeneration capabilities of South Africa shrublands. *Journal of Applied Ecology* 34, 317-332.
- Holmes, P., Newton, R.J., 2004. Patterns of seed persistence in South African Fynbos, *Plant Ecology* 172, 143-158.
- Holmes, P., Wood, J., Dorse, C., and Biodiversity Management Branch, 2008. City of Cape Town biodiversity report. Pp. 56-62.

- Holmes, P.M., 1989. Decay rates for buried *Acacia* seed populations of different density. *South African Journal of Botany* 55, 299-303.
- Holmes, P.M., 1990. Dispersal and predation in alien *Acacia*. *Oecologia* 83, 288-290.
- 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.
- Holmes, P.M., Richardson, D.M.; van Wilgen, B.W., Gelderblom, C., 2000. Recovery of South African fynbos vegetation following alien woody plant clearing and fire: implications for restoration. *Austral Ecology* 25, 631-639.
- Jeffrey, D.J., Holmes, P.M., Rebelo, A.G., 1988. Effects of dry heat on seed germination in selected indigenous and alien legume species in South Africa. *South African Journal of Botany* 54, 28-34.
- Keeley, J.E., 1987. Role of fire in seed germination of woody taxa in California chaparral. *Ecology* 68, 434-443.
- Keeley, J.E., Bond, W.J., 1997. Convergent seed germination in South Africa fynbos and Californian chaparral. *Plant ecology* 133, 153-167.
- Keeley, J.E., Bond, W.J., Bradstock, R.A., Pausas, J.G., Rundel, P.W., 2012. *Fire in Mediterranean Ecosystems: Ecology, Evolution and Management*. Cambridge University Press, New York.
- Keeley, J.E., Fotheringham, C.J., 1997. Trace gas emissions and smoke-induced seed germination. *Science* 276, 1248-1250.
- Keeley, J.E., Fotheringham, C.J., 1998. Smoke-induced seed germination in California chaparral. *Ecology* 79, 2320-2336.

- Kelly, E.F., Chadwick, O.A., Hilinski, T.E., 1998. The effect of plants on mineral weathering. *Biogeochemistry* 42, 139-43.
- Le Maître, D.C., 2004. Predicting invasive species impacts on hydrological processes: the consequences of plant physiology for landscape processes. *Weed Technology* 18, 1408-1410.
- Le Maître, D.C., Gaertner, M., Marchante, E., Ens, E.J., Holmes, P.M., Pauchard, A., O'Farrell, P.J., Rogers, A.M., Blanchard, R., Blignaut, J., Richardson, D.M., 2011. Impacts of invasive Australian acacias: implications for management and restoration. *Diversity and Distributions* 17, 1015–1029.
- Le Maître, D.C., Midgley, J.J., 1992. Plant reproductive. In: Cowling, R.M. (Ed.). *The Ecology of Fynbos: Nutrient, Fire and Diversity*. Cape Town, Oxford University press. pp. 135-174.
- Levitt, J., 1972. *Responses of plants to Environmental stresses full-stop*. Academic Press, New York.
- Light, M.E., Burger, B.V., Staerk, D., Kohout, L., van Staden, J., 2010. Butenolides from plant-derived smoke: natural plant growth regulators with antagonistic actions on seed germination. *Journal of Natural Products* 73,267–69.
- Lockwood, J.L., Hoopes, M.F., Marchetti, M.P., 2007. *Invasion Ecology*. Blackwell publishing Ltd, USA.
- Low, A.B., Rebelo, A.G., 1995. *Vegetation of South Africa, Lesotho and Swaziland*. Department of Environmental Affairs and Tourism, Pretoria. pp. 1-85.
- MacDougall, A.S., Turkington, R., 2005. Are the invasive species the drivers or passengers of change in degraded ecosystems? *Ecology* 86(1), 42-55.

- Marchante, E., Kjølner, A., Struwe, S., Freitas, H., 2009. Soil recovery after removal of the N₂-fixing invasive *Acacia longifolia*: consequences for ecosystem restoration. *Biological Invasions* 11, 813–823.
- Mehta, S., 2000. The invasion of South African fynbos by an Australian immigrant: The story of *Acacia saligna*. *Restoration and Reclamation Review* 6(5), 1-10.
- Midgley, G.F., Chapman, R.A., Hewitson, B., Johnston, P., de Wit, M., Ziervogel, G., Mukheibir, P., van Niekerk, L., Tadross, M., van Wilgen, B.W., Kgope, B., Morant, P.D., Theron, A., Scholes, R.J., Forsyth, G.G., 2005. *A Status Quo, Vulnerability and Adaptation Assessment of the Physical and Socio-economic Effects of Climate Change in the Western Cape*. Report to the Western Cape Government, Cape Town, South Africa. CSIR Report No. ENV-S-C 2005-073, Stellenbosch.
- Midgley, G.F., Hannah, L., Millar, D., Rutherford, M.C., Powrie, L.W., 2002. Assessing the vulnerability of species richness to anthropogenic climate change in a biodiversity hotspot. *Global Ecology and Biogeography* 11, 445-451.
- Midgley, S.J., Turnbull, J.W., 2003. Domestication and use of Australian acacias: Case studies of five important species. *Australian Systematic Botany* 16(1), 89-102.
- Morris, T.L., Esler, K.J., Barger, N.N., Jacobs, S.M., Cramer, M.D., 2011. Ecophysiological traits associated with the competitive ability of invasive Australian acacias. *Diversity and Distributions* 17, 898-910.
- Musil, C.F., 1993. Effect of invasive Australian acacias on the regeneration, growth and nutrient chemistry of South African lowland fynbos. *Journal of Applied Ecology* 30(2), 361-372.
- Musil, C.F., de Witt, D.M., 1991. Heat-stimulated germination in two Restionaceae species. *South African Journal of Botany* 57, 175-176.
- Mustart, P.J., Cowling, R.M., 1993. The role of regeneration stages in the distribution of edaphically restricted fynbos Proteaceae. *Ecology* 74, 1490–1499.

- Mustart, P.J., Rebelo, A.G., Juritz, J., Cowling, R.M., 2012. Wide variation in post-emergence desiccation tolerance of seedlings of Proteoid shrubs. *South African Journal of Botany* 80, 110-117.
- Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B., Kent, J., 2000. Biodiversity hotspots for conservation priorities. *Nature* 403, 853-858.
- Ooi, M.K.J., 2007. Comparative ecology of rare and common species in a fire-prone system, PhD thesis, School of Biological Sciences, University of Wollongong, <http://ro.uow.edu.au/theses/675/>.
- Pasquet-Kok, J., Creese, C., Sack, L., 2010. Turning over a new 'leaf': multiple functional significance of leaves versus phyllodes in Hawaiian *Acacia koa*. *Plant, Cell and Environment* 3, 2084-2100.
- Paynter, Q., Flanagan, G. J., 2004. Integrating herbicide and mechanical control treatments with fire and biological control to manage an invasive wetland shrub, *Mimosa pigra*. *Journal of Applied Ecology* 41, 615–629.
- Poynton, R.J., 2009. Tree planting in southern Africa, volume 3: Other genera. Department of Agriculture, Forestry and Fisheries, Pretoria, South Africa.
- Raimondo, D., von Staden, L., Foden, W., Victor, J.E., Helme, N.A., Turner, R.C., Kamundi, D.A., Manyama, P.A., 2009. Red List of South African Plants. *Strelitzia* 25. South African National Biodiversity Institute, Pretoria. pp. ix-668.
- Rebelo, A.G. 1996. Sand plain fynbos. In: Low, A.B., Rebelo, A.G., (Eds.). *Vegetation of South Africa, Lesotho and Swaziland*. Department of Environmental Affairs and Tourism, Pretoria. pp. 74.
- Rebelo, A.G., Boucher, C., Helme, N.A., Mucina, L., Rutherford, M.C., 2006. Fynbos biome. In: Mucina, L, Rutherford M.C., (Eds.). *The Vegetation of South Africa, Lesotho and Swaziland: Strelitzia*, 19, pp. 52–219.

- Richardson, D.M., Pyšek P., Rejmánek, M., Barbour, M.G., Panetta, F.D., West, C.J., 2000. Naturalization and invasion of alien plants: Concepts and definitions. *Diversity and Distributions* 6, 93-107.
- Rouget, M., Reyers, B., Jonas, Z., Desmet, P., Driver, A., Maze, K., Egoh, B., Cowling, R.M., 2004. South African National Spatial Biodiversity Assessment 2004: Technical report. Volume 1: Terrestrial component. South African National Biodiversity Institute, Pretoria.
<http://www.bcb.uwc.ac.za/pssa/articles/includes/NSBA%20Vol%201%20Terrestrial%20Component%20Draft%20Oct%2004.pdf>
- Royal Botanical Gardens KEW. (2008) Seed Information Database (SID), Version 7.1. Available at <http://data.kew.org/sid/> (May 2014).
- Ruwanza, S., Gaertner, M., Esler, K., 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, 132–141.
- Shaughnessy, G. 1980. Historical ecology of alien woody plants in the vicinity of Cape Town, South Africa. PhD thesis, University of Cape Town.
- Silvertown, J., Araya, Y.N., Linder, H.P., Gowing, D.J., 2012. Experimental investigation of the origin of fynbos plant community structure after fire. *Annals of Botany* 110, 1377-1383.
- Smith, R.E., van Wilgen, B.W., Forsyth, G.G., Richardson, D.M., 1992. Coexistence of seeders and resprouters in a fire-prone environment: the role of ecophysiology and soil moisture. In: van Wilgen, B.W., Richardson, D.M., Kruger, F.J., van Hensbergen, H.J., (Eds.). *Fire in South African mountain fynbos: Ecosystem, community and species response of Swartboskloof*. Springer-Verlag, Berlin Heidelberg.

- Stock, W.A., van der Heyden, F., Lewis, O.A.M., 1992. Plant structure and function. In: Cowling, R.M., (Ed.). *The Ecology of Fynbos: Nutrients, Fire and Diversity*, Oxford University Press, Cape Town.
- Strydom, M., Esler, K.J., Wood, A.R., 2012. *Acacia saligna* seed banks: sampling methods dynamics, Western Cape, South Africa. *South African Journal of Botany* 79, 140–147.
- Trabaud, L., Oustric, J., 1989. Heat requirements for seed germination of three *Cistus* species in the garrigue of southern France. *Flora* 183, 321-325.
- Turner, N.C., 1986. Adaptation to water deficits: a changing perspective. *Australian Journal of Plant Physiology* 13, 175-190.
- van de Venter, H.A., Esterhuizen, A.D., 1988. The effects of factors associated with fire on seed germination of *Erica sessiflora* and *E. hebecalyx* (Ericaceae). *South African Journal of Botany*, 54, 301-304.
- van Staden, J., Brown, N.A.C., Jager, A.K., Johnson, T.A., 2000. Smoke as a germination cue. *Plant Species Biology* 15, 167-178.
- van Wilgen, B., Richardson, D., Higgins, S., 2000. Integrated control of invasive alien plants in terrestrial ecosystems. In: Preston, G., Brown, G., van Wyk, E., (Eds.). *Best management practices for preventing and controlling invasive alien species, symposium proceedings. The Working for Water Programme*, Cape Town, South Africa. pp 118–128.
- van Wilgen, B.W., Bond, W.J., Richardson, D.M., 1992. Ecosystem management. In: Cowling, R.M., (Ed.). *The Ecology of Fynbos: Nutrients, Fire and Diversity*. Oxford University Press, Cape Town. pp. 345-371.
- van Wilgen, B.W., Richardson, D.M., 1985. The effects of alien shrub invasions on vegetation structure and fire behaviour in South African fynbos shrublands: A simulation study. *Journal of Applied Ecology* 22, 955-966.

- van Wilgen, B.W., van Hensbergen, H.J., 1992. Fuel properties of vegetation in Swartboskloof in Ecological Studies. In: van Wilgen, B.W., Richardson, D.M., Kruger, F.J., Van Hensbergen, H.J., (Eds.). Fire in South African Mountain Fynbos. Springer-Verlag, Berlin. pp. 38-53.
- van Wilgen, B.W.; Richardson, D.M.; Le Maître, D.C; Marais, C., Magadlela, D., 2001. The economic consequences of alien plant invasions: example of impacts and approaches to sustainable management in South Africa. *Environment, Development and Sustainability* 3, 145-168.
- Witkowski, E.T.F., 1991. Growth and competition between seedlings of *Protea repens* (L.) L. and the alien invasive, *Acacia saligna* (Labill.) Wendl. in relation to nutrient availability. *Functional Ecology* 5, 101-110.
- Yelenik, S.G., Stock, W.D., Richardson, D.M., 2004. Ecosystem level impacts of invasive *Acacia saligna* in the South African Fynbos. *Restoration Ecology* 12(1), 45-51.

Table 1.1: Names and seed characteristics of target species of Cape Flats Sand Fynbos used in the experiments (on the entire thesis) designed to give recommendations for improved germination in large-scale restoration projects. Superscript numbers used to refer to the reference source for germination cues.

Family	Species and naming authority	Common name	Growth form	Germination cues	Survival mode	Chapter
Asteraceae	<i>Chrysocoma ciliata</i> L.	Bitterbos	Perennial woody shrublet	Smoke	Reseeder	3&4
Asteraceae	<i>Metalasia densa</i> (Lam.) P.O. Karis	Blombos	Ericoid Shrub	^{1,2} Light/smoke/heat	Reseeder	2,3&4
Asteraceae	<i>Senecio elegans</i> L.	Wild cineraria	Annual herb	³ Warm and cold stratification	Reseeder	2,3&4
Asteraceae	<i>Seriphium incanum</i> (Thunb.) Pers.	Slangbos	Shrub	² Unknown	Reseeder	2
Asteraceae	<i>Ursinia anthemoides</i> (L.) Poir. Subsp. Anthemoides	Marigold	Annual herb	³ Warm and cold stratification	Reseeder	2,3&4
Ericaceae	<i>Erica mammosa</i> Salisb.	Nine-pin heath	Ericoid Shrub	³ Dry heat/low temperatures	Resprouter	2,3&4
Ericaceae	<i>Erica plumosa</i> Thunb	Wolheide	Ericoid Shrub	⁴ Heat/smoke	Reseeder	2,3&4
Haemodoraceae	<i>Wachendorfia multiflora</i> (Klatt) J.C. Manning and Goldblatt	Dwarf mirrorface	Geophyte	¹ Smoke	Resprouter	3&4
Iridaceae	<i>Babiana villosula</i>	Hairy	Geophyte	^{1,5} Variable	Resprouter	2
Iridaceae	<i>Watsonia meriana</i> (L.) Mill. Var. meriana	Suurkanol	Geophyte	^{1,5} Variable	Resprouter	2,3&4
Proteaceae	<i>Leucadendron salignum</i> P.J. Bergius	Knopbos	Proteoid shrub	^{3,5} Smoke/scarification/warm and cold stratification	Resprouter	2,3&4
Proteaceae	<i>Protea repens</i> (L.) L.	Common sugarbush	Proteoid shrub	³ Smoke	Serotinous reseeder	3&4
Proteaceae	<i>Protea scolymocephala</i> (L.) Reichard	Thistle sugarbush	Proteoid shrub	³ Smoke/hot water/scarification	Serotinous	2,3&4
Proteaceae	<i>Serruria fasciflora</i> Salisb. Ex Knight	Common pin spearhead	Proteoid shrub	⁵ Cold/fluctuating temperatures	Reseeder	2,3&4
Restionaceae	<i>Thamnochortus punctatus</i> Pillans	Dotty dangle reed	Restioid	^{6,7} Smoke and heat	Reseeder	2,3&4
Rhamnaceae	<i>Phylica cephalantha</i> Sond.	Sandveld hardleaf	Ericoid Shrub	³ Smoke	Resprouter	2,3&4
Rutaceae	<i>Agathosma imbricata</i> (L.) Willd	Boegoe	Ericoid Shrub	⁸ Scarification	Resprouter	2,3&4
Rutaceae	<i>Diosma oppositifolia</i> L.	Bitter buchu	Ericoid Shrub	⁵ Heat/ scarification/ fluctuating temperatures	Resprouter	2,3&4
Thymelaeaceae	<i>Passerina corymbosa</i> Eckl. ex C.H. Wright	Common gonna	Ericoid Shrub	^{2,3} Alternating temperature	Reseeder	2,3&4

¹Brown and Botha, 2004; ²Holmes and Newton, 2004; ³Cowell, 2013; ⁴Dixon et al., 1995; ⁵Cowling, 1992; ⁶Brown et al., 1994; ⁷Brown, 1992; ⁸Royal Botanical Gardens KEW, 2008

CHAPTER 2: IMPROVING GERMINATION OF 16 CAPE FLATS SAND FYNBOS SPECIES: APPLICATIONS OF PRE-GERMINATION TREATMENTS TO INFORM LARGE-SCALE RESTORATION PROGRAMMES.

ABSTRACT

Cape Flats Sand Fynbos (CFSF) is considered as critically endangered largely due to agricultural development, urban transformation, and degradation by invasive alien *Acacia* species. The City of Cape Town is clearing alien plants at Blaauwberg Nature Reserve (BBNR) in an attempt to restore one of the largest remaining fragments of CFSF. These efforts are associated with challenges, since alien stands have depleted indigenous soil-stored seedbanks and active restoration is required for establishing the major indigenous plant guilds and to restore areas that have been cleared of the alien plants. The aim of the study was to determine pre-germination treatments that can improve germinability of seeds of selected indigenous target species to be used in large-scale restoration projects.

Pre-germination treatments in seeds of 16 CFSF species were tested in a growth chamber. Seed viability and germinability (i.e. seed germination) were analysed. A comparison of different viability tests (i.e. x-ray, tetrazolium and cut test) indicated that the quickest test to estimate viability was the cut test. However, the cut test is not practical in the field especially when small seeds are being collected. Species showed varying responses to the different treatments with only the smoke water treatment having a consistent positive effect on seed germination for all functional groups. Only the annual, *Senecio elegans*, had a negative response to smoke water pre-germination treatment. A positive response was observed in *Thamnochortus punctatus* and *Erica plumosa* with the application of a combination of heat and smoke water. Another positive response was observed in *Babiana villosula* with the application of scarification as a pre-germination treatment. Additional analyses of days of germination (i.e. average germination period, germination lag phase, and duration) showed the effectiveness of pre-germination treatments in enhancing seed germination of species with short periods.

Pre-germination treatments should be applied to seeds of target species in restoration efforts to maximise germinability and establishment. This study shows that although pre-germination treatment responses are species-specific smoke water pre-germination treatment could be used for all functional groups. Other treatments can be applied for specific species.

Keywords: Germinability; Guild structure; Heat; Restoration; Smoke water

2.1. INTRODUCTION

Cape Flats Sand Fynbos is considered to be critically endangered and the main threat to this vegetation type is habitat transformation caused by cultivation and urban sprawl (Low and Rebelo, 1995; Rebelo, 1996; Rebelo et al., 2006), with invasive alien species being the second largest threat (i.e. Port Jackson (*Acacia saligna* (Labill.) Wendl.) and Rooikrans (*Acacia cyclops* A. Cunn. Ex G. Don)). Despite of large control efforts, there are challenges that hinder the process of restoring Sand Fynbos, even on cleared sites with a short history of dense invasion (Holmes, 2002; Holmes, 2008). Challenges to restoring fynbos include destroyed soil structure (Holmes, 2001), an increase in the total nitrogen pool (Yelenik et al., 2004), depleted indigenous seed banks (Holmes and Cowling, 1997, Holmes, 2002), secondary invasions that suppress and replace indigenous seedlings (Holmes et al., 2005) and the low emergence success of indigenous species (Cowell, 2013; Stuart Hall, unpublished data). Gaertner et al. (2011) concluded that in most cases active restoration would be required in order to assist indigenous species recovery and to successfully restore an area that was previously invaded by *Acacia* species. Active restoration can involve either planting of propagated material or direct sowing of seeds (Holmes et al., 2008; Ruwanza et al., 2013). Sowing seeds can be used to restore a diverse guild composition. However, to minimise risk of failure, seed germinability needs to be assessed using pre-germination treatments.

Since the targeted vegetation type is endangered, large-scale seed collection is difficult.

According to Cowell (2013), it is therefore important to collect viable seeds and to pre-treat them in order to break dormancy (physical, physiological, morphological or morphophysiological) (Baskin and Baskin, 2004) before sowing them to ensure germination success in the field. A dormant seed is one that does not have the capacity to germinate under combinations of normal environmental factors that are otherwise favourable for its germination (Baskin and Baskin, 2004). Since many seeds are dormant, it is important to understand how to overcome dormancy and facilitate germination (Baskin and Baskin, 2004).

A range of germination cues have been identified in fynbos species, including alternating temperature regimes (Brown and Botha, 2004), heat pulse to rupture the seed coat of hard-seeded species (Jeffrey et al., 1988), dry heat stimulating the seed embryo directly (Musil and De Witt, 1991) and high-temperature desiccation of the seed coat (Mustart et al., 2012). Additionally, the chemicals such as ethylene, ammonia, and Butenolides contained in smoke stimulate seed germination (Van de Venter and Esterhuizen, 1988; Light et al., 2010). Other cues include physical scarification of the seed coat mimicking heat pulse, scarification in the soil and combinations of treatments (Ooi, 2007; Cowell, 2013).

It is incontestable that in the fynbos, chaparral, Kwongan, and other Mediterranean-climate vegetation types, fire influences seed germination. However, only a few studies have tested different germination treatments for fynbos species targeted for restoration in lowland fynbos ecosystems (Brown, 1993; Brown and Botha, 2004; Cowell, 2013).

In this study, laboratory experiments were conducted to investigate various aspects of seed germinability and viability. The term 'seeds viability' 'is used to describe whether seed or seed populations are alive when given a specific viability test', whereas seed germinability refers to a seed or seed population having germinated, when given a germination test' (Gosling, 2003). This study's aim was to test different seed pre-germination treatments that could improve germinability in selected indigenous species that potentially may be used in large-scale restoration. The following key questions were posed: 1) which pre-germination treatments were the most effective for specific fynbos species (i.e. smoke water, heat, scarification, or combinations thereof). 2) Which pre-germination treatments yielded the highest germination response and rate among the species tested? 3) Which method (i.e. cut, tetrazolium, X-ray) was best in giving a quick estimate of viability prior to germination testing?

2.2. MATERIALS AND METHODS

2.2.1. Species collection, cleaning, storage and study site

The main fynbos plant guilds were selected, namely Restioid (wiry reed-like graminoids in the family Restionaceae); Ericoid (fine-leaved shrubs in the families Asteraceae, Ericaceae, Rhamnaceae, Rutaceae, Thymelaeaceae); Proteoid (larger shrubs of the family Proteaceae); annual forbs and geophyte (mainly winter ephemeral species in the families Amaryllidaceae, Colchicaceae, Haemodoraceae, and Iridaceae) (Table 1.1). Seeds were collected in Blaauwberg Nature Reserve and in natural vegetation remnants along the N7 road including the Friend's patch (a previously invaded site in BBNR that has recovered to a functional fynbos community) (McKay et al., 2005).

Seeds of 16 species were collected by hand between July 2012 and January 2013, when seeds were ripe and ready to be collected (Newton et al., 2002; P. M. Holmes personal communication). Phenological (flowering and seeding time) information was used to help select target species for restoration experiments as per Millennium Seed Bank Partnership (MSBP)'s standards of harvesting (Smith et al., 2004). Seeds were collected using various methods, including bagging plants, hand picking, stripping and collecting stems for ripening of seeds at room temperature. Seeds were collected in numbers from populations large enough to sustain annual collections (Hay and Smith, 2004; Smith et al., 2004).

Mature seeds were placed in paper bags and stored under dry and cold conditions with a relative humidity of 15% in 15° C in the Millennium Seed Bank project cold dry room storage in Kirstenbosch Botanical Gardens seed room (Buitink and Hoekstra, 2004).

Seed debris were cleaned by sieving using Seed Hand Test Sieves (Round Brass of varying sizes) before being aspirated using Zigzag aspirator (Selecta Industries Netherlands) to remove chaff (Cowell, 2013). After considering available literature on pre-germination treatments, seeds of 16 fynbos species were selected for germination testing (Table 1.1). For each test method, (i.e. germination and viability test (i.e. cut, x-ray, tetrazolium) 100 seeds (4×25 samples) per species were selected) (Table 1.1 and 2.1). Clean seeds were then weighed and packed in sealed paper bags and labelled with a batch number and species name. In this study, the term 'seed' refers to the plant's unit of dispersal/diaspora.

2.2.2. Experimental design

2.2.2.1. Viability experiment

To test seed viability a cut test, an X-ray test and the Tetrazolium (TZ) test was used, wherein four replicates of 25 seeds per plant species were used for each test.

The cut test is one of the simplest methods to estimate seed viability. In the cut test, seeds (4×25) were dissected with a scalpel under a dissecting microscope. The seeds that were full and partially full were categorised as good quality and those that were empty and decayed were categorised as poor quality.

Four replicates of 25 seeds per plant species were used for X-ray test to evaluate seed quality. Seed batches of plant guilds were sent to MSB head office, Wakehurst Place, Sussex in the United Kingdom. The seed fill was estimated for the samples of all species using Faxitron digital X-ray machine (Qados, Sandhurst, U.K.), set at the standard of Millennium Seed Bank settings (22kV and 0.3 mA for 20s) (Cowell, 2013). The X-ray method allows the detection of empty and partially filled seeds, seeds damaged by insects, and seeds with abnormally developed internal structures (Willan, 1985). The fill score was based on the presence or absence of fully formed embryo (structurally sound and looking all white inside).

The Tetrazolium (TZ) test (Moore, 1985) was done to get estimates of seed viability; with the TZ test, the staining of the seed gives an indication of viability (i.e. seeds that are stained red are categorised as viable whereas seeds that are white are considered as not viable). The materials needed for a TZ test included Petri dishes (for small seeds), beakers (for larger seeds), scalpel, forceps, microscope, medicine dropper/ plastic pipettes, dispensing bottle, 2,3,5-Triphenyltetrazolium chloride, and filter paper.

A tetrazolium concentration of 1.0% was prepared wherein one gram of the tetrazolium salt was dissolved in the distilled water to make a 100 ml solution. The pH of the solution has to be 7.3 for the proper staining to occur, since solutions of a pH lower than 4 would not stain even viable embryos. The solution was stored in a refrigerator in an amber coloured bottle to prevent reaction that can occur in the presence of light. Four replicates of 25 seeds per plant species were used for Tetrazolium salt test. Seed coats of hard-coated seeds were removed using a scalpel; this allowed the tetrazolium solution to penetrate the embryo, soft-coated seeds were soaked in tetrazolium without being dissected for 24 hours in room temperature.

After 24 hours soaking in 1.0% tetrazolium concentration at room temperature only *Senecio elegans* stained red (Personal observation). Temperature influences the staining reaction during soaking and it takes place twice as fast at 20-30°C (Moore, 1985).

A low temperature incubator (LTIM 40, Labcon, South Africa) with a temperature of $\pm 30^{\circ}\text{C}$ was therefore used to speed up the staining reaction. In the incubator some seeds began to stain between 24-96 hours of soaking wherein others seeds stained completely only after 168 hours of soaking with varying intensity (Lakon, 1949). The seeds were removed from the TZ solution as soon as they were stained and rinsed three times in water. Seeds were assessed under a microscope for the staining pattern in the embryo and the intensity of the staining according to the Tetrazolium Testing Handbook (AOSA, SCST, 2010). When viable the embryo was stained red, non-viable seeds had a whitish colour and those that were weak had a pinkish colour.

2.2.2.2. Germinability experiment

In all treatments including the control, 100 seeds per species were used, which were divided into four replicates of 25 seeds per treatment. According to the International Standards for Seed Testing (ISTA 1976), about 200 seeds should be used in a germination test. However, because of the limited number of seeds available from Cape Flats Sand Fynbos (CFSF) plant guilds 100 seeds per species were used in four replicates of 25 seeds each. Growth chambers and the equipment were disinfected with 70% ethanol to minimise contamination of seeds. The six pre-germination treatments were conducted separately and not all pre-germination treatments were applied to seeds of all species as precaution to avoid embryo damage due to seed size and thickness of the seed coat (personal observation) (Table 2.2). Pre-germination treatments applied were:

- a) Control (no treatment).
- b) Smoke water solution treatment: One standard concentration of 10% smoke solution was prepared with 50 ml “Liquid smoke plus”³ that was diluted with 450ml distilled water to make 500 ml using graduated cylinder. Seeds were treated using a freshly prepared batch of smoke solution by soaking them in 9 ml smoke water solution for 24 hours.
- c) Heat-pulse treatment: A constant temperature of 100 °C was maintained in a 240 litre, digital deluxe oven (Scientific, Model no. 278, Series 2000, South Africa). Seeds were placed in a 12-hole muffin tray with each hole having a replicate of 25 seeds to ensure that they all get the same 5 minutes exposure to the temperature in the oven. Then they were removed from the oven and left to cool off under room temperature.
- d) Scarification: Soil samples (coarse sand) were collected from the field (BBNR) and were sieved using a vibratory sieve shaker Analysette 3 Spartan (Fritsch, Germany). The sieved coarse sand was then autoclaved for 90 minutes at 121°C. Then 100 ml coarse sand was added into a 250 ml bottle containing a sandpaper (150 micrometre grit) duck tapped against the wall of the bottle. Seeds apportioned per species in replicates (4×25) were then added in the bottle with coarse sand and scarified by shaking them for 10 minutes.
- e) Smoke water solution-heat pulse combination: Seeds were exposed to 100 °C in an oven for 5 minutes as in c), and then smoke treated as in b).
- f) Smoke water solution-scarification combination: Seeds were scarified as in d) and then smoke treated as in b).

³ Liquid smoke plus- from Kirstenbosch Botanical Gardens is prepared by burning fresh fynbos plant material. The smoke is drawn from a smoke generator through drums (20-30 L) containing water for up to 60 minutes.

Polyethylene Petri dishes containing 2 discs of Whatman no.1 filter paper that was soaked in 1.5 ml 0.1% benlate solution to control fungi (Pierce et al., 1995) were used. After all the pre-germination treatments, 25 seeds were placed in each Petri dish ($n = 4$ replicates) allowing separation of the seeds to minimise fungal spread. Seeds were watered with 3 ml of distilled water. Filter paper was changed every two weeks to help control fungi and benlate and water was added as described above. Some Petri dishes at the upper level of the racks in the growth chamber dried faster than those at lower racks; hence, they were moved around after every 7 days. Each Petri dish was labelled with the species name of the seed being tested, treatment and replication number. Petri dishes were sealed in plastic polythene bags and closed with a clip (Keeley and Fotheringham, 1997; Holmes and Newton, 2004). Petri dishes were then transferred to the Low Temperature Germination Growth Chambers (Labcon, Model: LTGC-M-70, South Africa) in stacks of eight Petri dishes with alternating winter temperatures of 21° C /10° C and a photoperiod of 10 h L/14 h D using cool white Osram Duluxstar compact (11w≈60w) energy saving light bulbs (DST STICK 11 W/827 E14, United Kingdom). Winter temperatures were used because the main field germination season is in winter from April to August, whereas September is too late for germination and successful seedling establishment before the hot, dry summer (Enright and Lamont, 1989; Auld and O’Connell, 1991; van Wilgen et al., 1992; De Lange and Boucher, 1993; Bond and van Wilgen, 1996).

In this study, the term ‘germination rate’ refers to how quickly seed germination occurs over given periods and may be compared in cumulative germination curves. Germinability refers to the ability of the seeds to germinate. Germination period is defined as the average time in which particular seeds will germinate given optimum conditions. “Lag phase” refers to the number of days it takes for 10% of the seeds to germinate. “Duration of germination” refers to the time in which seeds will germinate at 90%. Germination was monitored by counting, recording, and removing germinated seeds on the 7th day and thereafter every 3rd day. Germination is defined as emergence of the radicle by 2 mm from the seed coat (Coops and Van der Velde, 1995; Bicksler, 2011). This germination experiments ran for a minimum of 8 weeks after which ungerminated seeds were soaked in tetrazolium solution (prepared as described above) to determine viability (International Seed Testing Association (ISTA), 1976).

2.2.3. Statistical analysis

All statistical analyses were run using the software package STATISTICA© version 12 (StatSoft, Inc.: Tulsa, Oklahoma 2014) with an Alpha (α)-level of 0.05. All data were tested for normality using both probability plots and normal expected frequency histograms. Viability experiments' (cut, x-ray, and tetrazolium) data were analysed using descriptive statistics to determine mean percentages and Standard Deviation. Results of the cut test were compared with results of x-ray and tetrazolium tests respectively using Wilcoxon matched pairs test to determine whether the practical cut test adequately reflects quality and viability.

Data was checked for normality using normal probability plots including a test of for homogeneity using Levene's Test for Homogeneity of Variances. A mixed model Analysis of Variance was used to analyse germination response to treatment with the output of biplots and cumulative percentage germination curves. Wherein, in the Variance Estimation and Precision test, an alternative to ANOVA estimation, was provided by restricted maximum likelihood estimation (REML) this was used to generate cumulative plots (pre-germination treatments vs time) per species. The variability in germination percentage within each species was estimated by calculating the coefficient of variation, which is defined as the ratio of the standard deviation to the mean. To determine the efficacy of the different pre-germination treatments in breaking dormancy and enhancing germinability, percentage germination data were used. One-way Analysis of Variance (ANOVA) was used to compare the effects of the different pre-germination treatments on the fynbos species. Where ANOVAs were significant, a Post hoc Fisher LSD test was then used to determine the difference between pre-germination treatments at $P < 0.05$, additionally Games-Howell post hoc test was used (where variances were unequal).

Ordination analysis (Principle component Analysis (PCA)) was used to determine variance in species response to different treatments. The amount of variance (the spread of data values) in the samples was determined using biplots. Species were plotted according to their germination rate along with the overall response of all species to the different treatments. Observations of how certain data points cluster together and whether specific pre-germination treatment variables were more common among those clusters were then made.

The average germination period in days was calculated using a formula $\frac{\Sigma(gt)}{\Sigma(g)}$ wherein g is the number of seeds germinating each day and t is the number of days between initiation of the first and the last seed germinating. The germination lag phase (days to 10% germination) and duration of germination (days to 90% germination) were calculated from the raw data tables (Jeffrey et al., 1988). The statistical analyses for average germination period, lag phase and duration of germination were run using 1-way ANOVAs.

2.3. RESULTS

2.3.1. Comparison of cut test with x-ray and cut test with tetrazolium test

For the quality and viability test, the overall data of the full seeds for x-ray and cut test were included, excluding the partially empty seeds, whilst the data of viable seeds for tetrazolium test were included, excluding the weakly seeds. There was a significant difference (Wilcoxon, $Z=4.87$; $P=0.001$) between results of the number of viable seeds for the cut test (with high number of filled seeds) and tetrazolium test (with low number of viable seeds) (Figure 2.1. a). There was no significant difference between the number of filled seeds of the x-ray and the cut test (Wilcoxon, $Z= 1.52$; $P<0. 05$; Figure 2.1. b), with the cut test being the most practical test compared to x-ray.

In the viability test species that were found having high quality (>60%) seeds using cut test and x-ray were *Watsonia meriana*, *Ursinia anthemoides*, *Senecio elegans*, *Babiana villosula*, *Metalasia densa*, *Passerina corymbosa* and *Agathosma imbricata* (Table 2.1). The species found to have low quality (<59%) seeds using the cut test were *Erica plumosa* whilst *Protea scolymocephala*, *Diosma oppositifolia* and *Serruria fasciflora* were found to have low quality seeds using x-ray. The species having the greatest percent of viable seeds in the study were *Watsonia meriana*, *Agathosma imbricata*, *Passerina corymbosa*, and *Babiana villosula* (Table 2.1). With the exception of these few species, seed viability was relatively low and the lowest being (*Erica plumosa* (Table 2.1).

There was a significant difference between the number of filled seeds in cut test and viable seeds in tetrazolium test in *Metalasia densa*, *Senecio elegans*, *Seriphium incanum*, and *Thamnochortus punctatus*. The number of seeds determined as viable by cut test was higher than those that were found to be viable by the tetrazolium test (Table 2.1). However, there was a similar response pattern between the two tests for *Agathosma imbricata*, *Babiana villosula* and *Watsonia meriana*, showing a high response, and *Erica plumosa*, *Diosma oppositifolia*, *Protea scolymocephala* and *Serruria fasciflora*, showing a low response.

2.3.2. Overall germinability in response to pre-germination treatments

The overall effects of the applied pre-germination treatments varied considerably among species with patterns following post-fire survival strategies. Species with low germinability were *Diosma oppositifolia*, *Phylica cephalantha*, *Protea scolymocephala*, *Serruria fasciflora* and *Erica plumosa* whilst in *Agathosma imbricata*, *Babiana villosula*, *Senecio elegans*, and *Watsonia meriana* germinability was relatively high, with over 60% germination recorded in all the pre-germination treatments (Table 2.3).

Of the 16 species tested, nine showed a positive response to smoke water treatment (Table 2.3). Smoke water, the only treatment applied to all species, increased germination totals, but this increase was significant relative to the control only in two species: *Seriphium incanum* and *Ursinia anthemoides*. In contrast, there was a significant decrease on *Senecio elegans* relative to control.

There was a significant and negative effect of heat and a combination of heat and smoke water on germinability of *Metalasia densa*, which was significantly less than smoke water and control. Heat and a combination of heat and smoke water was significantly lower than control, smoke water, scarification and a combination of scarification and smoke water pre-germination treatment for *Babiana villosula* (Table 2.3). A significant positive response to the combination of heat and smoke water treatment was recorded for *Erica plumosa* and for *Thamnochortus punctatus* (Table 2.3). There was a negative impact of heat and a combination of heat and smoke water pre-germination treatment relative to control in *Passerina corymbosa* (Table 2.3).

Of the five species tested, four species showed a positive response, but only one was significant to the scarification pre-germination treatment (Table 2.3), *Babiana villosula*. The effect of physical scarification of the seed coat yielded negative germination responses in some species. In *Leucadendron salignum* scarification alone was ineffective, but scarification in combination with smoke water induced germination significantly more than the control.

The ordination analysis was performed separately for the different species grouped according to the treatment received (see Table 2.2). The first Principal Component Analysis (PCA) included the following pre-germination treatments: smoke water, heat, heat- smoke water combination and control (Figure 2.2 a) for the species *Erica plumosa*, *Thamnochortus punctatus* Pillans, *Passerina corymbosa*, *Metalasia densa* and *Erica mammosa*. The total variation explained on PC1 was 51% and 40% on PC2. *Erica plumosa* and *Thamnochortus punctatus* clustered at the combination of heat and smoke water pre-germination treatment which shows that the species reacted similarly and positively to that treatment compared to smoke water pre-germination treatment. The position of *Passerina corymbosa* and *Metalasia densa* close to the control and smoke water pre-germination treatment (Figure 2.2. a; Table 2.3.) indicates their positive response to smoke (although not significant) response to these treatments compared to a negative response to heat and a combination of heat and smoke water pre-germination treatment.

The second PCA included the pre-germination treatments smoke water and control (Figure 2.2 b) for the species *Seriphium incanum*, *Watsonia meriana*, *Serruria fasciflora*, *Senecio elegans* and *Ursinia anthemoides*. The total variation explained on PC1 was 76% and 19% on PC2. *Seriphium incanum* and *Ursinia anthemoides* clustered towards smoke water pre-germination treatment. *Senecio elegans* is clustered towards control (Figure 2.2. b), which could mean that there was no positive response with smoke water pre-germination treatment. *Watsonia meriana* and *Serruria fasciflora* had a positive response to smoke water although it was not significantly different from control (Table 2.3).

The third PCA included the pre-germination treatments control, smoke water, scarification, heat, scarification-smoke water and combination of heat and smoke water (Figure 2.2 c) for the species *Agathosma imbricata*, *Diosma oppositifolia*, *Babiana villosula*, *Protea scolymocephala*, *Phylica cephalantha*. The total variation explained on 34% of PC1 and 24% of PC2 and the species were distributed across all the pre-germination treatments which means that there was a positive response of species to all treatments, although it was not significantly different (Table 2.3). *Babiana villosula* had a positive response to smoke water, scarification and a combination of scarification and smoke water (although not significantly different from control (Table 2.3)) and a negative response to heat and a combination of heat and smoke water pre-germination treatment (Table 2.3).

2.3.3. Effects of pre-germination treatments on germination Rate

The cumulative germination curves (Figure A.1.a-p) indicate that for *Thamnochortus punctatus* Pillans (Figure A.1. n); *Erica plumosa* (Figure A.1. e); *Agathosma imbricata* (Figure A.1. a) and *Phylica cephalantha* (Figure A.1. i) germination response rate was faster when heat and smoke water were combined over smoke water alone. *Senecio elegans* (Figure A.1. k); *Diosma oppositifolia* L. (Figure A.1. c) and *Watsonia meriana* (Figure A.1. p) showed no improvement in germination rate following pre-germination treatments as compared to the control. *Serruria fasciflora* (Figure A.1. m); *Erica mammosa* (Figure A.1. d); *Seriphium incanum* (Figure A.1. l); *Metalasia densa* (Figure A.1. g) and *Ursinia anthemoides* (Figure A.1. o) showed a faster germination response to smoke water compared to the control. *Leucadendron salignum* (Figure A.1. f) had a faster response when scarification and smoke water pre-germination treatments were combined, compared to the control.

2.3.4. Effects of pre-germination treatments on average germination period, lag phase, duration of germination

Germinability results (Table 2.3) for *Passerina corymbosa* and *Seriphium incanum* were significant, but the germination period (Table 2.4) was not significantly different (slower in all treatments). The germinability results for *Agathosma imbricata* and *Leucadendron salignum* (Table 2.3) were not significantly different (in all treatments), but germination lag phase (Table 2.5), was significantly different (faster in a combination of scarification and smoke water and a combination of heat and smoke water). There were significant differences in the average germination period with faster response to smoke water in *Phyllica cephalantha* and *Erica mammosa*, and a faster response to a combination of scarification and smoke water and that of heat and smoke water in *Agathosma imbricata*; and faster response to a combination of scarification and smoke water in *Leucadendron salignum* (Table 2.4), with significant differences in germinability (Table 2.3).

There were significant differences in *Agathosma imbricata* (faster response to a combination of scarification and smoke water pre-germination treatment) and *Leucadendron salignum* (faster response to a combination of scarification and smoke water and that of heat and smoke water combination pre-germination treatment) for the duration of germination (Table 2.6) despite no significant differences for germinability (Table 2.3). There were no significant differences in the germination lag phase (Table 2.5) of *Passerina corymbosa*, *Seriphium incanum* and *Ursinia anthemoides* when there were significant differences for germinability (Table 2.3). There were no significant differences in the average germination period (Table 2.4) of *Passerina corymbosa* and *Seriphium incanum* despite significant differences for germinability (Table 2.3). There were no significant differences in the duration of germination (Table 2.6) for *Passerina corymbosa*, *Senecio elegans* and *Seriphium incanum* when there were significant differences in germinability (Table 2.3). *Metalasia densa* and *Thamnochortus punctatus* (faster response in smoke water pre-germination treatment), and *Senecio elegans* (faster response in control) (Table 2.5), had a significant difference in their germination lag phase between treatments when there was no significant differences in smoke water treatment in their germinability (Table 2.3).

2.4. DISCUSSION

2.4.1. Comparison of quality and viability tests

The standard for judging seed viability is always a germination test under ideal conditions. Information on ideal germination conditions is scarce in hyper-diverse systems such as those found in the Cape Floristic Region, so this often has to be inferred from a general understanding of the ecology of the species. Crucial to understanding any aspect of seed ecology is being able to determine whether a seed is viable and dormant or non-viable (Ooi, 2007). There was no difference between cut and the x-ray test with similar estimates of seed quality for all species. A dominant principle of a cut test is that if there is a doubt about a seed being ‘filled’ then it must be classified as ‘dead’ or non-viable. It means that the evaluation of a cut test tends to be biased. According to Gosling (2003), the consequences of the visual classification and interpretation of seeds will affect the seed collection in the field, with immature collection as a result thereof. The tetrazolium test gave a lower viability result (although not on all species) than the cut and x-ray tests, which means that the latter two overestimate viability. This could be an issue of ‘embryo-less’ seeds that develop an endosperm to occupy the seed cavity (Gosling, 2003), or the fact that they were immature at time of collection (Newton et al., 2002). The tetrazolium viability test is best in estimating potential germinability. However, it is tedious and time-consuming. Hence the cut-test is a quick, cheap method to get a reasonable “ball-park” prediction of germinability. This is practical in restoration applications which have to balance the need for accuracy against the need for convenience.

2.4.2. Germination responses

Species can have dormant seeds that require different germination cues depending on their morphology and dormancy mechanisms (Baskin and Baskin, 2004; Ooi, 2007). For example, hard-seeded species fail to imbibe water unless the seed coat barrier is broken by heat or other types of scarification.

The effectiveness of the pre-germination treatments in enhancing seed germination was highlighted by the relationships between average germination period, lag phase and the duration of germination in response to the various pre-germination treatments. Germination responses of the 16 species varied, and depended on the direct and indirect fire-related germination cues applied as pre-germination treatments. *Metalasia densa* (although not significantly different from control), *Ursinia anthemoides* and *Seriphium incanum* responded positively to smoke water treatment which is in line with other research findings on Asteraceae (Brown and Botha, 2004). These obligate reseeder species responded positively to smoke chemicals. It appears that the active compounds in smoke act as a major cue for germination in some species and probably interact with heat, incubation temperature, and light as cues for germination (Brown et al., 1993; Brown et al., 1994). Plant-derived smoke is known to include both germination promoter and germination inhibitor volatiles (van Staden et al., 2004). Smoke may have a positive effect on the seeds that are morphologically and chemically dormant, because smoke increases the sensitivity of seeds to the hormones that promote embryo growth (van Staden et al., 2000).

Heat inhibited the germination of *Metalasia densa*, confirming results obtained by Pierce (1990). Both studies conflict with results from Musil (1991), who reported an increase in germination from 9.1-52.5%, following exposure of *Metalasia densa* to a heat treatment of 100 °C for 6 minutes. This is due to the different temperatures and duration of exposure to heat treatment.

Thamnochortus punctatus showed a positive response when heat and smoke water pre-germination treatments were combined, but no response when heat and smoke water treatments were applied separately. Brown et al. (1994) tested 32 species of Restionaceae for response to smoke and found that germination improved for 25 of these. They reported that seeds of *T. punctatus* gave a highly significant response to smoke treatment (Brown et al., 1994). Musil (1991) reported that seeds of *T. punctatus* failed to respond to heat treatment (Brown 1993). The reason for the conflicting results could be that different exposure periods (temperature and time) were used in my study compared to a study by Musil (1991), or that there is variation in germination response depending on where and when the seeds are collected. My results imply that there are species in the Restionaceae family that respond to a combination of treatments, yet further research is needed to further explicate the exposure periods.

Erica plumosa responded positively when heat and smoke water pre-germination treatments were combined. However, seed viability was low. According to the findings of Brown and Botha (2004), out of the 53 species tested about 33 (62%) *Erica* species responded positively to smoke yet there were species that had a low germination rate. Brown and Botha (2004) suggested that species with a low germination rate might require another germination treatment; as indicated here for *Erica plumosa*.

Dry heat has been shown to improve germination in Rutaceae, Rhamnaceae, Ericaceae, and Restionaceae (Musil, 1991). However, heat pulse and a combination of heat and smoke water pre-germination treatments applied to *Agathosma imbricata* (Rutaceae) and *Phyllica cephalantha* (Rhamnaceae) yielded no positive response. Germination may have been negatively affected by the 5 minutes exposure to 100°C. A temperature range of ca. 80-100°C has been suggested to initiate maximum germination response in other fynbos taxa (Musil, 1991) with some species even achieving maximum germination response at 120°C. However, results by Jeffrey et al, (1988) suggest that the intensity of heat may not be as important as the duration of heat treatments. Different heat pulse temperatures and durations should be tested for these plant families.

Some species' seeds germinated without pre-germination treatment. These species might respond to changes in light, which is commonly induced by removal of vegetation cover by fire and soil disturbance. In other species, germination may be triggered by alternating diurnal temperatures (Keeley, 1994). Species like *Senecio elegans* had a high response to no treatment, with lowest response to smoke water treatment. These results are in line with the findings of Brown and Botha (2004) who suggest that germination could be triggered by a favourable temperature regime.

Other species that required no treatment to germinate included *Passerina corymbosa*, which responded negatively to heat pre-germination treatments. Brown and Botha (2004) also found that some Thymelaeaceae species had a significant positive response to smoke and others responded best to no treatment. The geophyte *Watsonia meriana* germinated well without any pre-germination treatment, which is understandable given that the family Iridaceae do not have seeds with long-term persistence (Holmes and Newton, 2004). These results are in agreement with those of Brown et al. (2003) who showed that geophytes exhibited a very low response to smoke and Keeley and Bond (1997) showed that geophytes lacked fire-stimulated germination, whilst the majority of annuals were chemically stimulated by smoke. *Babiana villosula* showed a positive response to scarification.

In general, low germination rates were recorded for most of the species tested on the percentage germination (with the exception of, *Agathosma imbricata*, *Seriphium incanum*, *Leucadendron salignum*, *Babiana villosula*, *Senecio elegans*, *Ursinia anthemoides*, and *Watsonia meriana* with germinability above 60%). Most non-germinated seeds were found to be non-viable which could be due to poor seed collection (Newton et al., 2002) or perhaps seed viability was lost during storage (Newton et al., 2006).

The germination period (i.e. average germination rate, germination lag phase and duration of germination) reflected the results on the germinability described above. Species that responded positively to smoke treatment also responded more rapidly after having been given a smoke treatment (i.e. average germination period, germination lag phase and duration of germination were shorter than in any other treatment). The seeds of species which showed a high percentage of germination to a combination of heat and smoke pre-germination treatment were *Erica plumosa*. *Agathosma imbricata* (the greatest germination was observed following the heat treatment), also responded faster i.e. average germination period, germination lag phase and duration of germination were shorter than in any other treatment.

The overall results for all species show that smoke water pre-germination treatment yielded the highest germination rate on the cumulative curves and germination lag phase, although higher germination rate was observed in other treatments, they were not as significant to some species. The response to all pre-germination treatments was however species-specific. The study clearly shows the importance of pre-germination treatment to accelerate the rate of CFSF species germination, which is best, combined with favourable winter temperature conditions (Brits, 1986; Bond and van Wilgen, 1996).

2.4.3 Management implications for large-scale restoration programmes

Although the tetrazolium test provides an accurate assessment of viability for seeds, the amount of time for staining can take up to 168 hours (Moore, 1985). The cut test is used in many studies since it is cheaper, simpler, and faster than the x-ray and tetrazolium tests (Gosling, 2003). The limitation is that it overestimates viability of fynbos species. Therefore, it is recommended that cut test be used in the field for ease of seed collection with the verification of laboratory tetrazolium test,. However, a cut test is only practical in the field when collecting large seeds where it can help seed collectors to estimate the proportion of damaged, empty, and mature seeds and therefore to estimate quantities needed. In general, it is recommended that seeds should be collected when they are naturally dispersing wherever possible.

Care should be taken in storing seeds in a cool, dry, insect free facilities since exposure to inappropriate conditions can damage the collection. Finally, the time of collection should be carefully considered in relation with the phenology of the species in the field. Seeds that are not fully developed when collected can account for poor seed germination. Seeds should be sown in autumn, when field conditions to prime germination are appropriate ahead of the winter rains.

This study only tested a handful of the many hundreds of CFSF species but gives insight on potential species to use in large-scale restoration and the required pre-germination treatments to use. In light of this study, restoration efforts that use direct seeding should first study targeted species' dormancy-breaking requirements prior to sowing seeds. Results of the germination study of 16 CFSF species show that seeds of most species do require pre-germination treatment in order to enhance germination. As anticipated, about half the species responded to fire-related cues (i.e. smoke water and heat), which reflects findings to date for fynbos species (Brown et al., 1993; van Staden et al., 2000). Species' responses to pre-germination treatments were species-specific and restoration applications should budget for some species-specific treatment. As most of the species, with the exception of *Senecio elegans* were positively enhanced by smoke, it is recommended that smoke pre-germination treatment be applied to seeds of all species. Smoke can be applied as water (i.e. smoke water) or in the gaseous form to seed trays, field soil, directly applied to seeds or applied to a combination of both seeds and soil (dealt with in Chapter 3) and there is an optimum level of concentrations of smoke required that affects seed germination. The heat pulse might have damaged seeds of some species. This treatment should therefore only be applied to species for which a positive response to heat treatment has been established. Further research is needed on optimal heat pulse intensity and duration. Suitable pre-germination treatments for the selected target species for restoration are given in Table 2.7.

Species suitable for large-scale restoration will fulfil the following requirements: one of the missing structural guilds, easy to collect, good germination response following identified pre-germination treatment. Furthermore, replication of structural guilds is recommended to build resilience in the restored community (Waller et al., 2015). In general, species that germinate without treatments or those species that have the potential to respond positively to pre-germination treatments are the best candidates for active restoration interventions via seeding, as they are likely to recruit more successfully.

In summary, for, large-scale restoration efforts involving re-seeding, pre-germination treatments are essential, but as shown by the large variation in species responses, treatments are likely to be species-specific. It is therefore important to conduct viability and germination trials prior to restoration.

REFERENCES

- AOSA, SCST (Association of Official Seed Analysts, Society of Commercial Seed Technologists), 2010. Tetrazolium Testing Handbook. AOSA, SCST, Ithaca, New York.
- Auld, T.D., O'Connell, M.A., 1991. Predicting patterns of post-fire germination in 35 eastern Australian Fabaceae. *Australian Journal of Ecology* 16, 53-70.
- Baskin, C.C., Baskin, J.M., 2004. Germinating seeds of wildflowers, an ecological perspective. *Hortechology* 14(4), 467-473.
- Bicksler, A.J., October 2011. Testing seed viability using simple germination tests, ECHO Asia notes-A regional supplement to ECHO development notes. Issue 11, 1-20.
- Bond, W.J., van Wilgen, B.W., 1996. Fire and plants. 1st edition. Chapman and Hall, London.
- Brown, N.A.C., 1993. Promotion of fynbos seeds by plant-derived smoke. *New Phytologist* 123, 575-583.
- 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, 559-581.
- Brown, N.A.C., Jamieson, H., Botha, P.A., 1994. Stimulation of germination in South African species of Restionaceae by plant-derived smoke. *Plant Growth Regulation* 15, 93-100.
- Buitink, J., Hoekstra, F.A., 2004. Understanding and predicting optimal storage conditions and longevity: a biophysical approach. In: Smith, R., Dickie, J., Linington, S., Pritchard, H., Probert, R., (Eds.). *Seed Conservation: Turning Science into Practice*. London, Kew Publishing.
- Coops, H., van der Velde, G., 1995. Seed dispersal, germination and seedling growth of six helophyte species in relation to water-level zonation. *Freshwater Biology* 34, 13-20.
- Cowell, C., 2013. Investigating the most favourable seed establishment methods of restoring sand fynbos on old fields. Unpublished Master of Technology thesis, Cape Peninsula University of Technology.

- De Lange, J.H., Boucher, C., 1990. Autecological studies on *Audouinia capitata* (Bruniaceae). 1. Plant derived smoke as a seed germination cue. *South African Journal of Botany* 55, 700-703.
- Enright, N.J., Lamont, B.B., 1989. Seed banks, fire season, safe sites and seedling recruitment in five co-occurring *Banksia* species. *Journal of Ecology* 77, 1111-22.
- Gaertner, M., Richardson, D.M., Privett, S.D.J., 2011. Effects of alien plants on ecosystem structure and functioning and implications for restoration: insights from three degraded sites in South African fynbos. *Environmental Management* 48(1), 57-69.
- Gosling, P.G., 2003. Viability testing. In: Smith, R.D, Dickie, J.B, Linington, S.L., Pritchard, H.W., Probert, R.J., (Eds.) *Seed conservation turning science into practice*. Royal Botanical Gardens, Kew. pp. 445–481.
- Hay, F.R., Smith, R.D., 2004. A Field Manual for Seed Collectors. In Smith, R., Dickie, J., Linington, S., Pritchard, H., Probert, R., (Eds.). *Seed Conservation: Turning Science into Practice*. London, Kew Publishing.
- Holmes, P., 2001. Shrubland restoration following woody alien invasion and mining: effects of topsoil depth, seed source, and fertilizer addition. *Restoration Ecology* 9(1), 71-84.
- Holmes, P., 2002. Depth distribution and composition of seed banks in alien-invaded and uninvaded Fynbos vegetation. *Australian Ecology* 27, 110-120.
- Holmes, P., 2008. Optimal ground preparation treatments for restoring lowland fynbos vegetation on old fields. *South African Journal of Botany* 74, 33-40.
- Holmes, P., Cowling, R.M., 1997. The effects of invasion by *Acacia Saligna* on the guild structure and regeneration capabilities of South Africa shrublands. *Journal of Applied Ecology* 34, 317-332.
- Holmes, P., Newton, R.J., 2004. Patterns of seed persistence in South African Fynbos. *Plant Ecology* 172, 143-158.
- Holmes, P.M., Richardson, D.M., Esler, K.J., Witkowski, E.T.F., Fourie, S., 2005. A decision-making framework for restoring riparian zones degraded by invasive alien plants in South Africa. *South African Journal of Science* 101, 553–564.

- International Seed Testing Association (ISTA) 1976. International Rules for seed testing. Rules and annexes. International Seed Testing Association, Seed Science and Technology 4, 3–177.
- Jeffrey, D.J., Holmes, P.M., Rebelo, A.G., 1988. Effects of dry heat on seed germination in selected indigenous and alien legume species in South Africa. South African Journal of Botany 54, 28-34.
- Keeley, J.E., Bond, W.J., 1997. Convergent seed germination in South African fynbos and Californian chaparral. Plant ecology 133, 153-167.
- Keeley, J.E., Fotheringham, C.J., 1997. Trace gas emissions and smoke-induced seed germination. Science 276, 1248-1250.
- Keeley, J.E., Fotheringham, C.J., 1998. Smoke-induced seed germination in California chaparral.
- Lakon, G., 1949. The topographical tetrazolium method for determining the germinating capacity of seeds. Plant Physiology 24(3), 389-394.
- Light, M.E., Burger, B.V., Staerk, D., Kohout, L., Van Staden, J., 2010. Butenolides from plant-derived smoke: natural plant-growth regulators with antagonistic actions on seed germination. Journal of Natural Products 73, 267–69.
- Low, A.B., Rebelo, A.G., 1995. Vegetation of South Africa, Lesotho and Swaziland. Department of Environmental Affairs and Tourism, Pretoria. pp. 1-85.
- McKay, J.K., Christian, C.E., Harrison, S., Rice, K., 2005. “How local is local?” A review of practical conceptual issues in the genetics of restoration. Restoration Ecology 13, 432-440.
- Moore, R.P., 1985. Handbook on tetrazolium testing. Tetrazolium Committee of the International Seed Testing Association. The International Seed Testing Association, Zurich, Switzerland. pp.9-19
- Musil, C.F., 1991. Seed bank dynamics in sand plain lowland fynbos. South African Journal of Botany 57, 131-142.
- Musil, C.F., De Witt, D.M., 1991. Heat-stimulated germination in two Restionaceae species. South African Journal of Botany 57, 175-176.

- Mustart, P.J., Rebelo, A.G., Juritz, J., Cowling, R.M., 2012. Wide variation in post-emergence desiccation tolerance of seedlings of Proteoid shrubs. *South African Journal of Botany* 80, 110-117.
- Newton, R.J., Bond, W.J., Farrant, J.M., 2002. Seed development, morphology, and quality testing in selected species of the nut-fruited Restionaceae. *South African Journal of Botany* 68, 226-230.
- Ooi, M.K.J., 2007. Comparative ecology of rare and common species in a fire-prone system, PhD thesis, School of Biological Sciences, University of Wollongong, <http://ro.uow.edu.au/theses/675>.
- Pierce, S.M., Esler, K., Cowling, R.M., 1995. Smoke-induced germination of succulents (Mesembryanthemaceae) from fire prone and fire-free habitats in South Africa. *Oecologia* 102, 520-522.
- Rebelo, A.G., 1996. Sand plain fynbos. In: Low, A.B. and Rebelo, A.G. (Eds.). *Vegetation of South Africa, Lesotho and Swaziland*. Department of Environmental Affairs and Tourism, Pretoria. pp.74.
- Rebelo, A.G., Boucher, C., Helme, N.A., Mucina, L., Rutherford, M.C., 2006. Fynbos biome. In: Mucina, L, Rutherford M.C. (Eds.). *The Vegetation of South Africa, Lesotho and Swaziland: Strelitzia*, 19, pp. 52–219.
- 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, 132–141.
- Smith, P.P., Wyk, E.V., 2004. The Millennium Seed Bank Project in South Africa: a review. In: Smith, R., Dickie, J., Linington, S., Pritchard, H., Probert, R., (Eds.). *Seed Conservation: Turning Science into Practice*. London, Kew Publishing.
- StatSoft Inc., 2014. STATISTICA (Data Analysis Software System), version 12. www.statsoft.com. Tulsa, Oklahoma.
- van de Venter, H.A., Esterhuizen, A.D., 1988. The effects of factors associated with fire on seed germination of *Erica sessiflora* and *E. hebecalyx* (Ericaceae). *South African Journal of Botany* 54, 301-304.

- van Wilgen, B.W., Bond, W.J., Richardson, D.M., 1992. Ecosystem management. In: Cowling, R.M. (Ed.). *The Ecology of Fynbos: Nutrients, Fire and Diversity*. Oxford University Press, Cape Town. pp. 345-371.
- 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, 62-38.
- Yelenik, S.G., Stock, W.D., Richardson, D.M., 2004. Ecosystem level impacts of invasive *Acacia saligna* in the South African Fynbos. *Restoration Ecology* 12(1), 45-51.

Table 2.1: The most practical tests in estimating quality and viability on seventeen target indigenous fynbos species tested using three test: cut, x-ray and tetrazolium test. Mean percentages \pm SD; $n=4 \times 25$, for the seed quality and viability tests. Cut and tetrazolium test results of T-test arcsine transformed percentage are compared for their variability (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS is not significant $P > 0.05$). Test with the highest quality % for each species is indicated in bold.

Species name	Cut test				X-ray test				Tetrazolium test				t-value
	Full	Partially empty	Empty	Decayed	Full	Partially empty	Empty	Damaged/infested by insects	Viable	Weak	Non-viable	Empty	
<i>A. imbricata</i>	100 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	92 \pm 10.83	4 \pm 5.66	3 \pm 3.83	1 \pm 2.00	98 \pm 2.31	2 \pm 2.31	0 \pm 0	0 \pm 0	1.73 ^{NS}
<i>B. villosula</i>	80 \pm 15.66	1 \pm 2.00	9 \pm 8.87	10 \pm 9.52	89 \pm 2.00	0 \pm 0	4 \pm 3.27	7 \pm 3.83	81 \pm 3.83	0 \pm 0	0 \pm 0	19 \pm 3.83	-0.12 ^{NS}
<i>D. oppositifolia</i>	44 \pm 3.27	29 \pm 6.83	22 \pm 6.93	5 \pm 3.83	18 \pm 11.55	22 \pm 9.52	60 \pm 7.30	0 \pm 0	29 \pm 10.00	19 \pm 12.38	25 \pm 8.87	27 \pm 8.87	2.72 ^{NS}
<i>E. mammosa</i>	74 \pm 12.44	13 \pm 6.83	5 \pm 7.57	8 \pm 8.00	59 \pm 16.12	13 \pm 11.02	16 \pm 10.33	12 \pm 5.66	51 \pm 20.75	17 \pm 8.25	17 \pm 14.74	15 \pm 3.83	2.16 ^{NS}
<i>E. plumosa</i>	6 \pm 5.16	6 \pm 5.16	38 \pm 30.38	50 \pm 24.55	36 \pm 11.31	12 \pm 15.32	51 \pm 25.59	1 \pm 2.00	15 \pm 6.00	0 \pm 0	0 \pm 0	85 \pm 6.00	-2.38 ^{NS}
<i>L. salignum</i>	58 \pm 15.49	5 \pm 3.83	14 \pm 13.66	23 \pm 3.83	49 \pm 8.25	2 \pm 4.00	46 \pm 8.33	3 \pm 3.83	36 \pm 11.78	8 \pm 4.62	8 \pm 3.27	48 \pm 12.65	1.84 ^{NS}
<i>M. densa</i>	97 \pm 3.83	3 \pm 3.83	0 \pm 0	0 \pm 0	72 \pm 6.53	7 \pm 8.25	19 \pm 8.25	2 \pm 4.00	52 \pm 19.87	10.00 \pm 5.16	33 \pm 22.72	5 \pm 7.57	3.84*
<i>P. corymbosa</i>	99 \pm 2.00	0 \pm 0	1 \pm 2.00	0 \pm 0	77 \pm 11.02	5 \pm 3.83	18 \pm 7.66	0 \pm 0	89 \pm 9.45	6 \pm 12.00	4 \pm 5.66	1 \pm 2.00	1.89 ^{NS}
<i>P. cephalantha</i>	73 \pm 11.02	5 \pm 5.03	15 \pm 6.00	7 \pm 3.83	68 \pm 8.64	9 \pm 2.00	19 \pm 8.87	4 \pm 0	50 \pm 21.04	11 \pm 14.38	16 \pm 7.30	23 \pm 8.87	2.11 ^{NS}
<i>P. scolymocephala</i>	35 \pm 18.58	0 \pm 0	28 \pm 11.78	37 \pm 6.83	2 \pm 2.31	0 \pm 0	95 \pm 2.00	3 \pm 3.83	35 \pm 5.03	0 \pm 0	0 \pm 0	65 \pm 5.03	0.00 ^{NS}
<i>S. elegans</i>	84 \pm 13.47	7 \pm 3.83	1 \pm 2.00	8 \pm 13.47	75 \pm 47.37	0 \pm 0	1 \pm 2.00	24 \pm 48.00	24 \pm 7.30	34 \pm 13.66	40 \pm 5.66	2 \pm 4.00	6.04**
<i>S. incanum</i>	91 \pm 3.83	3 \pm 2.00	0 \pm 0	6 \pm 5.16	93 \pm 5.03	3 \pm 3.83	2 \pm 4.00	2 \pm 2.31	55 \pm 10.00	13 \pm 6.00	14 \pm 6.93	18 \pm 9.52	5.69*
<i>S. fasciflora</i>	24 \pm 8.64	7 \pm 3.83	62 \pm 4.00	7 \pm 6.83	17 \pm 10.52	4 \pm 3.27	47 \pm 16.12	32 \pm 14.24	24 \pm 8.64	0 \pm 0	7 \pm 2.00	69 \pm 8.87	0.00 ^{NS}
<i>T. punctatus</i>	47 \pm 7.57	6 \pm 4.00	38 \pm 7.66	9 \pm 3.83	55 \pm 11.02	0 \pm 0	45 \pm 11.02	0 \pm 0	26 \pm 10.07	2 \pm 2.31	4 \pm 3.27	68 \pm 8.64	3.09*
<i>U. anthemoides</i>	61 \pm 19.97	29 \pm 17.09	10 \pm 5.16	0 \pm 0	99 \pm 2.00	0 \pm 0	1 \pm 2.00	0 \pm 0	51 \pm 10.52	11 \pm 6.00	33 \pm 7.57	5 \pm 3.83	1.35 ^{NS}
<i>W. meriana</i>	81 \pm 19.43	14 \pm 9.52	5 \pm 10.00	0 \pm 0	100 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	94 \pm 2.31	0 \pm 0	0 \pm 0	6 \pm 2.31	-1.24 ^{NS}

Table 2.2: List of pre-germination treatments methods based on available literature for Cape Flats Sand Fynbos species selected for use in large-scale restoration projects. X indicates treatments undertaken and Dash (–) indicates no pre-germination treatment applied.

Species	Control	Heat	Smoke	Scarification	Heat-smoke	Scarification-smoke
<i>Metalasia densa</i>	X	X	X	-	X	-
<i>Seriphium incanum</i>	X		X	-	-	-
<i>Ursinia anthemoides</i>	X		X	-	-	-
<i>Senecio elegans</i>	X		X	-	-	-
<i>Erica mammosa</i>	X	X	X	-	X	-
<i>Erica plumosa</i>	X	X	X	-	X	-
<i>Babiana villosula</i>	X	X	X	X	X	X
<i>Watsonia meriana</i>	X		X	-	-	-
<i>Serruria fasciflora</i>	X		X	-	-	-
<i>Protea scolymocephala</i>	X	X	X	X	X	X
<i>Leucadendron salignum</i>	X	X	X	X	X	X
<i>Thamnochortus punctatus</i>	X	X	X	-	X	-
<i>Phylica cephalantha</i>	X	X	X	X	X	X
<i>Diosma oppositifolia</i>	X	X	X	X	X	X
<i>Agathosma imbricata</i>	X	X	X	X	X	X
<i>Passerina corymbosa</i>	X	X	X	-	X	-

Table 2.3: Effects of different pre-germination treatments on 16 target indigenous fynbos species tested under growth chamber conditions (with alternating temperatures of 21° C /10° C and a photoperiod of 10h L/14hD). Data are means of germinants percentage \pm SE $n= 4 \times 25$ seeds and results of 1-way ANOVA arcsine transformed percentage are shown (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS is not significant $P > 0.05$). Superscript letters show where the significant differences lie within species between the treatments after post hoc Fisher LSD test. Treatment with the highest germination % for each species is indicated in bold. Dash (-) indicates no data collected.

Species names	Control	Smoke water	Scarification	Heat	Scarification and smoke water	Heat and smoke water	F	Sig.
<i>Agathosma imbricata</i>	62.00 \pm 4.76	75.00 \pm 6.81	75.00 \pm 9.57	83.00 \pm 5.26	75.00 \pm 4.43	76.00 \pm 2.83	$F_{(5,18)}=1.285$	NS
<i>Babiana villosula</i>	88.00 \pm 1.63 ^a	77.00 \pm 5.97 ^b	91.00 \pm 2.52 ^a	0 \pm 0 ^c	87.00 \pm 5.00 ^{ab}	0 \pm 0 ^c	$F_{(5,18)}=170.78$	***
<i>Diosma oppositifolia</i>	13.00 \pm 4.12	7.00 \pm 1.91	8.00 \pm 4.32	5.00 \pm 1.91	6.00 \pm 2.00	1 \pm 1.00	$F_{(5,18)}=1.933$	NS
<i>Erica mammosa</i>	44.00 \pm 5.89	44.00 \pm 1.63	-	37.00 \pm 3.79	-	42.00 \pm 2.58	$F_{(3,12)}=0.749$	NS
<i>Erica plumosa</i>	0 \pm 0 ^b	3.00 \pm 1.00 ^b	-	3.00 \pm 1.00 ^b	-	19.00 \pm 3.42 ^a	$F_{(3,12)}=21.905$	***
<i>Leucadendron salignum</i>	35.00 \pm 10.25	56.00 \pm 2.83	49.00 \pm 11.70	59.00 \pm 5.26	70.00 \pm 9.59	60.00 \pm 11.89	$F_{(5,18)}=1.651$	NS
<i>Metalasia densa</i>	44.00 \pm 5.16 ^{ab}	48.00 \pm 10.07 ^a	-	32.00 \pm 2.31 ^b	-	14.00 \pm 2.58 ^c	$F_{(3,12)}=6.657$	**
<i>Passerina corymbosa</i>	45.00 \pm 4.73 ^a	43.00 \pm 4.12 ^a	-	9.00 \pm 1.91 ^b	-	7.00 \pm 4.43 ^b	$F_{(3,12)}=25.721$	***
<i>Phyllica cephalantha</i>	30.00 \pm 7.57	21.00 \pm 8.06	16.00 \pm 4.00	20.00 \pm 5.16	28.00 \pm 4.32	34.00 \pm 7.39	$F_{(5,18)}=1.192$	NS
<i>Protea scolymocephala</i>	13.00 \pm 3.79	20.00 \pm 2.83	16.00 \pm 5.89	3.00 \pm 3.00	28.00 \pm 4.32	19.00 \pm 10.25	$F_{(5,18)}=2.186$	NS
<i>Senecio elegans</i>	83.00 \pm 6.40 ^a	27.00 \pm 5.51 ^b	-	-	-	-	$F_{(1,6)}=43.963$	***
<i>Seriphium incanum</i>	38.00 \pm 5.77 ^b	70.00 \pm 10.13 ^a	-	-	-	-	$F_{(1,6)}=7.529$	*
<i>Serruria fasciflora</i>	23.00 \pm 4.12	28.00 \pm 4.32	-	-	-	-	$F_{(1,6)}=0.701$	NS
<i>Thamnochortus punctatus</i>	0 \pm 0 ^b	1.00 \pm 1.00 ^b	-	0 \pm 0 ^b	-	39.00 \pm 4.12 ^a	$F_{(3,12)}=83.111$	***
<i>Ursinia anthemoides</i>	14.00 \pm 1.15 ^b	92.00 \pm 2.83 ^a	-	-	-	-	$F_{(1,6)}=651.86$	***
<i>Watsonia meriana</i>	98.00 \pm 1.15	89.00 \pm 7.19	-	-	-	-	$F_{(1,6)}=1.528$	NS

Table 2.4: The effect of different pre-germination treatment on the average seed germination period of target CFSF species. Data are mean days \pm SE $n= 4 \times 25$ seeds and results of 1-way ANOVA are shown (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS is not significant $P > 0.05$). Superscript letters show where the significant differences lie within species between the treatments after Fisher LSD and Games-Howell post hoc test. Dash (-) indicates no data collected.

Species name	Control	Smoke water	Scarification	Heat	Scarification-smoke water	Heat-smoke water	F	P
<i>Agathosma imbricata</i>	48.58 \pm 1.07 ^b	49.60 \pm 1.28 ^{ab}	52.27 \pm 1.11 ^a	47.09 \pm 0.33 ^b	32.63 \pm 2.28 ^c	30.36 \pm 0.83 ^c	F _(5,18) =53.798	***
<i>Babiana villosula</i> ¹	52.37 \pm 0.64 ^a	51.26 \pm 0.78 ^a	52.33 \pm 0.75 ^a	0 \pm 0 ^c	38.29 \pm 0.93 ^b	0 \pm 0 ^c	F _(5,18) =1616.7	**
<i>Diosma oppositifolia</i>	41.75 \pm 6.50	38.384 \pm 3.92	36.60 \pm 12.26	35.25 \pm 11.90	28.50 \pm 2.40	10.75 \pm 10.75	F _(5,18) =1.609	NS
<i>Erica mammosa</i>	33.24 \pm 0.73 ^a	23.15 \pm 0.89 ^b	-	33.01 \pm 2.20 ^a	-	30.87 \pm 1.53 ^a	F _(3,12) =10.561	**
<i>Erica plumosa</i>	0 \pm 0 ^b	45.00 \pm 15.02 ^a	-	41.25 \pm 13.80 ^a	-	39.57 \pm 1.53 ^a	F _(3,12) =4.254	*
<i>Leucadendron salignum</i>	22.16 \pm 1.41 ^{cb}	21.24 \pm 0.69 ^{cb}	25.08 \pm 1.09 ^a	23.52 \pm 0.52 ^{ab}	18.04 \pm 0.21 ^d	20.22 \pm 0.38 ^{cd}	F _(5,18) =8.981	**
<i>Metalasia densa</i>	56.65 \pm 0.98 ^a	43.60 \pm 1.71 ^b	-	56.23 \pm 0.61 ^a	-	40.48 \pm 3.49 ^b	F _(3,12) =17.223	**
<i>Passerina corymbosa</i>	45.11 \pm 1.02	39.44 \pm 2.44	-	40.44 \pm 3.72	-	36.45 \pm 12.24	F _(3,12) =0.303	NS
<i>Phylica cephalantha</i>	30.98 \pm 0.93 ^{ab}	25.73 \pm 2.37 ^b	34.90 \pm 1.58 ^a	35.03 \pm 3.42 ^a	32.20 \pm 2.81 ^{ab}	37.41 \pm 2.04 ^a	F _(5,18) =3.092	*
<i>Protea scolymocephala</i> ¹	28.38 \pm 0.94	26.17 \pm 0.91	27.11 \pm 1.12	14.50 \pm 14.50	21.25 \pm 0.49	24.94 \pm 8.47	F _(5,18) =0.554	NS
<i>Senecio elegans</i>	12.71 \pm 0.60	18.46 \pm 1.91	-	-	-	-	F _(1,6) =8.202	NS
<i>Seriphium incanum</i>	44.54 \pm 2.05	40.97 \pm 1.69	-	-	-	-	F _(1,6) =1.805	NS
<i>Serruria fasciflora</i>	51.38 \pm 1.97	51.89 \pm 1.53	-	-	-	-	F _(1,6) =0.043	NS
<i>Thamnochortus punctatus</i> ¹	0 \pm 0 ^b	8.50 \pm 8.50 ^{ab}	-	0 \pm 0 ^b	-	37.15 \pm 2.54 ^a	F _(3,12) =15.783	**
<i>Ursinia anthemoides</i>	26.44 \pm 4.98	7.60 \pm 0.30	-	-	-	-	F _(1,6) =14.25	NS
<i>Watsonia meriana</i>	17.79 \pm 0.21	16.42 \pm 1.41	-	-	-	-	F _(1,6) =0.914	NS

Table 2.5: The effect of different pre-germination treatments and number of days on the germination lag phase of germination (expressed as days for 10% germination. Data are mean days \pm SE $n= 4 \times 25$ seeds and results of 1-way ANOVA are shown (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS is not significant $P > 0.05$). Superscript letters show where the significant differences lie within species between the treatments after Fisher LSD and Games-Howell post hoc test (species that had this test indicated by superscript number). Dash (-) indicates no data collected.

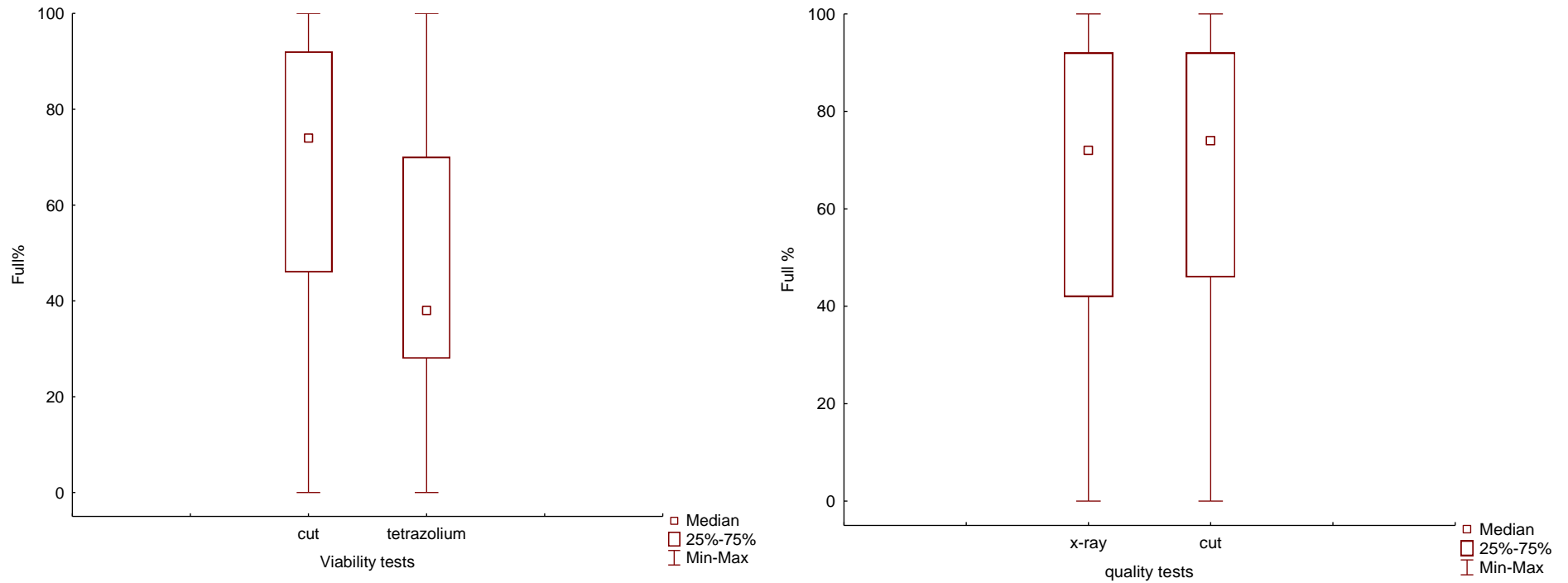
Species name	Control	Smoke water	Scarification	Heat	Scarification-smoke water	Heat-smoke water	F	P
<i>A. imbricata</i> ¹	29.00 \pm 3.32 ^{ab}	32.75 \pm 4.19 ^{ab}	39.75 \pm 4.37 ^{ab}	27.75 \pm 1.18 ^a	20.50 \pm 0.87 ^b	19.00 \pm 0.00 ^b	F _(5,18) =7.205	**
<i>B. villosula</i> ¹	38.50 \pm 1.50 ^a	32.00 \pm 2.12 ^{ab}	38.75 \pm 2.66 ^{ab}	0 \pm 0 ^c	26.00 \pm 0.71 ^b	0 \pm 0 ^c	F _(5,18) =137.09	***
<i>D. oppositifolia</i>	35.50 \pm 6.06	30.25 \pm 3.33	33.00 \pm 11.62	32.25 \pm 11.23	25.75 \pm 2.25	10.75 \pm 10.75	F _(5,18) =1.137	NS
<i>E. mammosa</i> ¹	18.75 \pm 1.89	16.00 \pm 0.00	-	20.50 \pm 1.94	-	19.00 \pm 0.00	F _(3,12) =1.923	NS
<i>E. plumosa</i>	0 \pm 0 ^b	45.00 \pm 15.02 ^a	-	41.25 \pm 13.80 ^a	-	34.00 \pm 3.00 ^a	F _(3,12) =3.976	*
<i>L. salignum</i> ¹	17.50 \pm 0.87 ^{ab}	15.50 \pm 0.50 ^b	17.25 \pm 0.95 ^{ab}	19.00 \pm 0.00 ^a	13.00 \pm 0.00 ^b	19.00 \pm 0.00 ^a	F _(5,18) =16.754	***
<i>M. densa</i>	44.75 \pm 4.75 ^a	21.75 \pm 2.10 ^b	-	49.00 \pm 4.06 ^a	-	31.75 \pm 2.84 ^b	F _(3,12) =12.01	***
<i>P. corymbosa</i> ¹	22.75 \pm 3.54	15.50 \pm 2.06	-	31.75 \pm 1.89	-	31.25 \pm 12.00	F _(3,12) =1.457	NS
<i>P. cephalantha</i>	20.50 \pm 0.87	21.75 \pm 1.70	26.00 \pm 4.95	22.00 \pm 2.12	22.00 \pm 1.22	19.75 \pm 1.89	F _(5,18) =0.744	NS
<i>P. scolymocephala</i> ¹	26.50 \pm 1.50	22.50 \pm 0.29	25.00 \pm 1.22	14.50 \pm 14.50	19.00 \pm 0.00	21.75 \pm 7.78	F _(5,18) =0.409	NS
<i>S. elegans</i> ¹	7.00 \pm 0.00	11.50 \pm 0.87	-	-	-	-	F _(1,6) =27.00	NS
<i>S. incanum</i>	23.50 \pm 2.87	20.25 \pm 4.40	-	-	-	-	F _(1,6) =0.382	NS
<i>S. fasciflora</i>	39.25 \pm 4.31	43.25 \pm 3.25	-	-	-	-	F _(1,6) =0.549	NS
<i>T. punctatus</i> ¹	0 \pm 0 ^b	8.50 \pm 17.00 ^{ab}	-	0 \pm 0 ^b	-	24.25 \pm 1.50 ^a	F _(3,12) =7.181	**
<i>U. anthemoides</i>	8.00 \pm 1.00	7.00 \pm 0.00	-	-	-	-	F _(1,6) =1.00	NS
<i>W. meriana</i> ¹	13.00 \pm 0.00	10.00 \pm 0.00	-	-	-	-	F _(1,6) =65535	NS

Table 2.6: Means \pm SE shows the effect of different pre-germination treatment on the germination duration (expressed as days for 90% germination) of sixteen target CFSF species. Data are means \pm SE $n=4 \times 25$ seeds and results of 1-way ANOVA percentage are shown (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS is not significant $P > 0.05$). Superscript letters show where the significant differences lie within species between the treatments after Fisher LSD and Games-Howell post hoc test (species that had this test indicated by superscript number). Dash (-) indicates no data collected

<i>Species name</i>	Control	smoke water	scarification	heat	scarification-smoke water	heat-smoke water	F-value	P-value
<i>A. imbricata</i> ¹	56.50 \pm 0.87 ^a	57.25 \pm 0.75 ^a	58.00 \pm 0.00 ^a	54.25 \pm 1.44 ^a	48.25 \pm 6.86 ^{ab}	41.50 \pm 1.94 ^b	F _(5,18) =4.669	*
<i>B. villosula</i> ¹	58.00 \pm 0.00 ^a	57.25 \pm 0.75 ^a	58.00 \pm 0.00 ^a	0 \pm 0 ^b	51.25 \pm 2.25 ^a	0 \pm 0 ^b	F _(5,18) =902.84	***
<i>D. oppositifolia</i>	46.00 \pm 7.04	43.75 \pm 6.17	35.25 \pm 11.90	38.25 \pm 12.77	28.00 \pm 2.12	10.75 \pm 10.75	F _(5,18) =1.955	NS
<i>E. mammosa</i>	51.25 \pm 1.89 ^a	25.75 \pm 1.44 ^c	-	43.75 \pm 3.09 ^b	-	38.50 \pm 1.94 ^b	F _(3,12) =24.347	***
<i>E. plumosa</i>	0 \pm 0 ^b	45.00 \pm 15.02 ^a	-	41.25 \pm 13.80 ^a	-	38.50 \pm 1.94 ^a	F _(3,12) =4.186	*
<i>L. salignum</i> ¹	24.25 \pm 2.25 ^b	23.50 \pm 0.87 ^b	31.75 \pm 4.48 ^a	23.50 \pm 0.87 ^b	19.75 \pm 0.75 ^b	22.75 \pm 0.75 ^b	F _(5,18) =3.454	*
<i>M. densa</i> ¹	58.25 \pm 0.25 ^a	57.25 \pm 0.75 ^a	-	58.00 \pm 0 ^a	-	45.25 \pm 4.64 ^b	F _(3,12) =7.169	**
<i>P. corymbosa</i>	57.25 \pm 0.75	51.25 \pm 4.80	-	43.00 \pm 6.12	-	37.00 \pm 12.37	F _(3,12) =1.488	NS
<i>P. cephalantha</i>	36.25 \pm 5.66	26.50 \pm 1.94	35.50 \pm 0.87	37.75 \pm 5.66	37.75 \pm 5.66	47.25 \pm 3.09	F _(5,18) =2.384	NS
<i>P. scolymocephala</i> ¹	29.50 \pm 1.50	26.50 \pm 0.87	28.00 \pm 1.22	14.50 \pm 14.50	22.00 \pm 0	29.25 \pm 9.78	F _(5,18) =0.652	NS
<i>S. elegans</i>	24.25 \pm 7.28	19.00 \pm 3.00	-	-	-	-	F _(1,6) =0.444	NS
<i>S. incanum</i>	52.00 \pm 5.05	55.75 \pm 1.44	-	-	-	-	F _(1,6) =0.510	NS
<i>S. fasciflora</i>	54.25 \pm 1.89	53.50 \pm 1.50	-	-	-	-	F _(1,6) =0.097	NS
<i>T. punctatus</i> ¹	0 \pm 0 ^b	8.50 \pm 8.50 ^b	-	0 \pm 0 ^b	-	45.75 \pm 3.82 ^a	F _(3,12) =21.956	***
<i>U. anthemoides</i>	26.50 \pm 6.65	9.25 \pm 0.75	-	-	-	-	F _(1,6) =6.640	NS
<i>W. meriana</i>	19.75 \pm 0.75	18.25 \pm 0.75	-	-	-	-	F _(1,6) =2.000	NS

Table 2.7: A summary table of the study species and their recommended pre-germination treatments to be considered for restoration programmes.

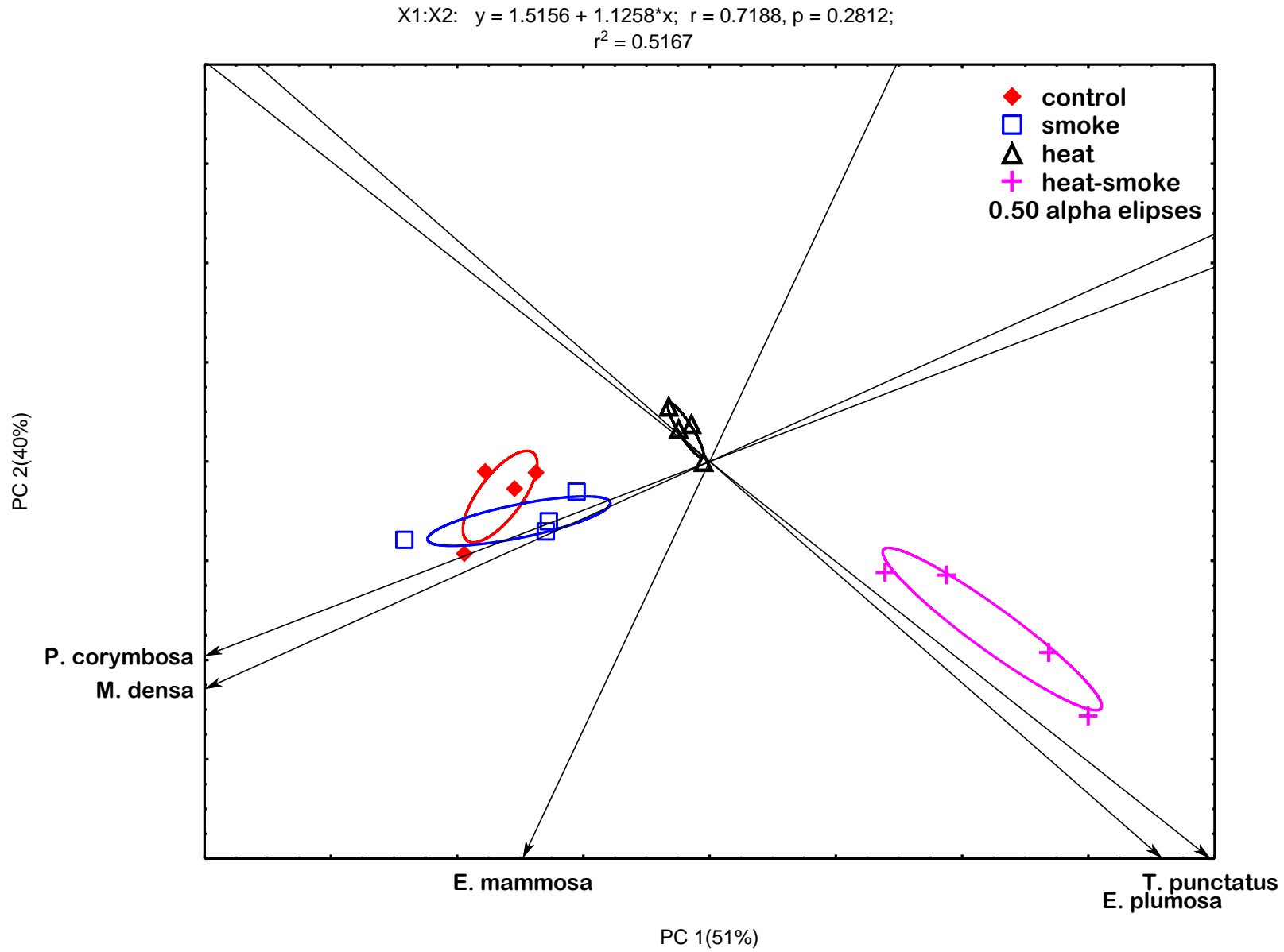
Species names	Recommended pre-germination treatment
<i>Agathosma imbricata</i>	None, not inhibited by smoke treatment
<i>Babiana villosula</i>	Smoke water/scarification or combination
<i>Erica mammosa</i>	None, but not inhibited by heat
<i>Erica plumosa</i>	Smoke water and heat combination
<i>Leucadendron salignum</i>	None, but not inhibited by smoke, heat or scarification
<i>Metalasia densa</i>	None, but not inhibited by smoke water
<i>Passerina corymbosa</i>	None, but not inhibited by smoke water
<i>Phylica cephalantha</i>	None, but not inhibited smoke water, scarification or heat
<i>Protea scolymocephala</i>	None, not inhibited by smoke water, scarification
<i>Senecio elegans</i>	None
<i>Seriphium incanum</i>	Smoke water
<i>Thamnochortus punctatus</i>	Smoke water and heat combination
<i>Ursinia anthemoides</i>	Smoke water
<i>Watsonia meriana</i>	None, not inhibited by smoke water



a) Viability test for cut and tetrzolium test

b) Quality test for x-ray and cut test

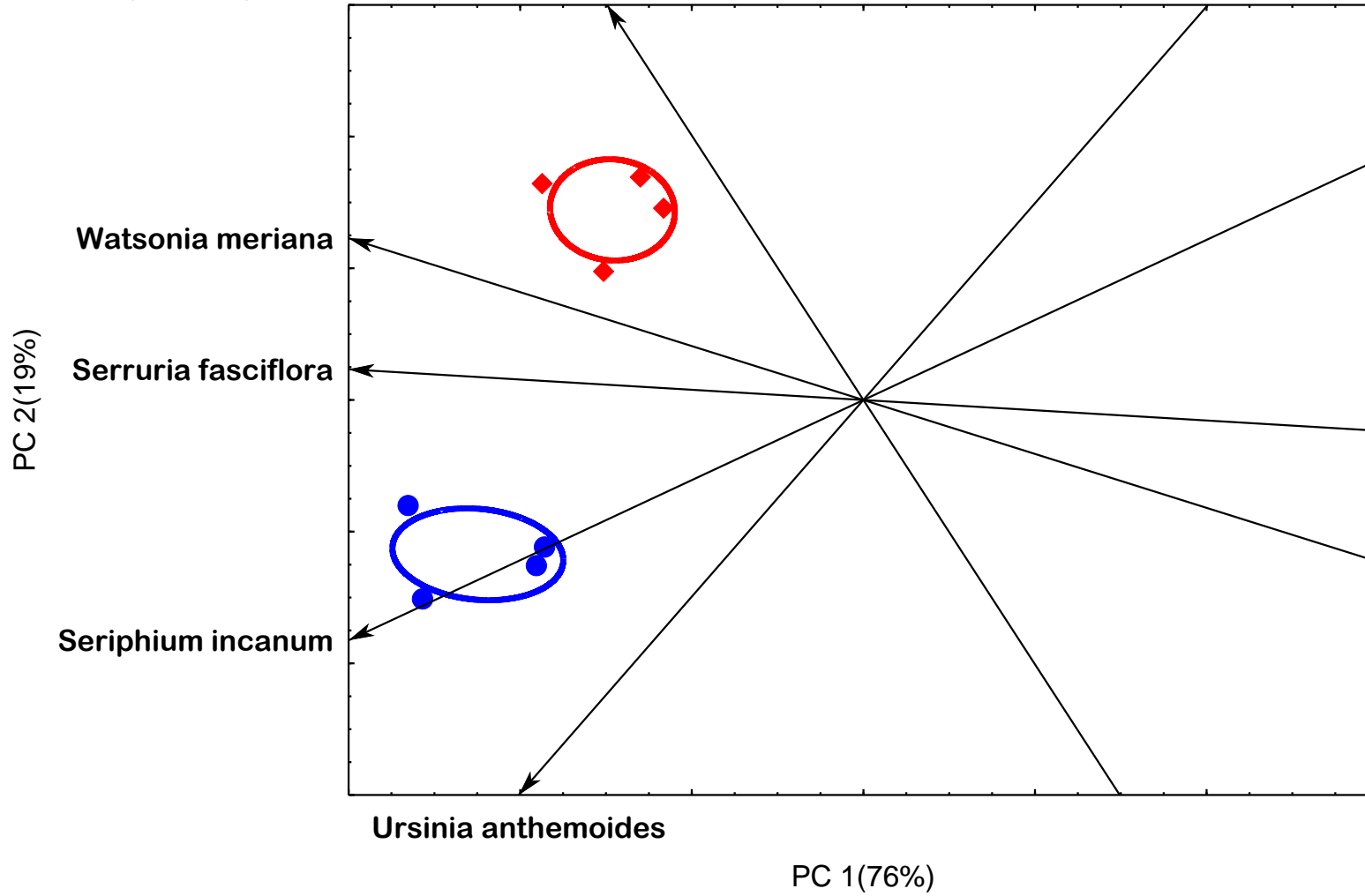
Figure 2.1: Box and Whisker plot showing median numbers of full seeds of 16 CFSF target species tested for the most practical test in estimating a) seed viability using cut and tetrzolium test and, b) seed quality using cut and x-ray test. The results will be used to give restoration ecologists an idea of the suitable quality and viability test of seed during and after collection or before doing more complex seed germination.



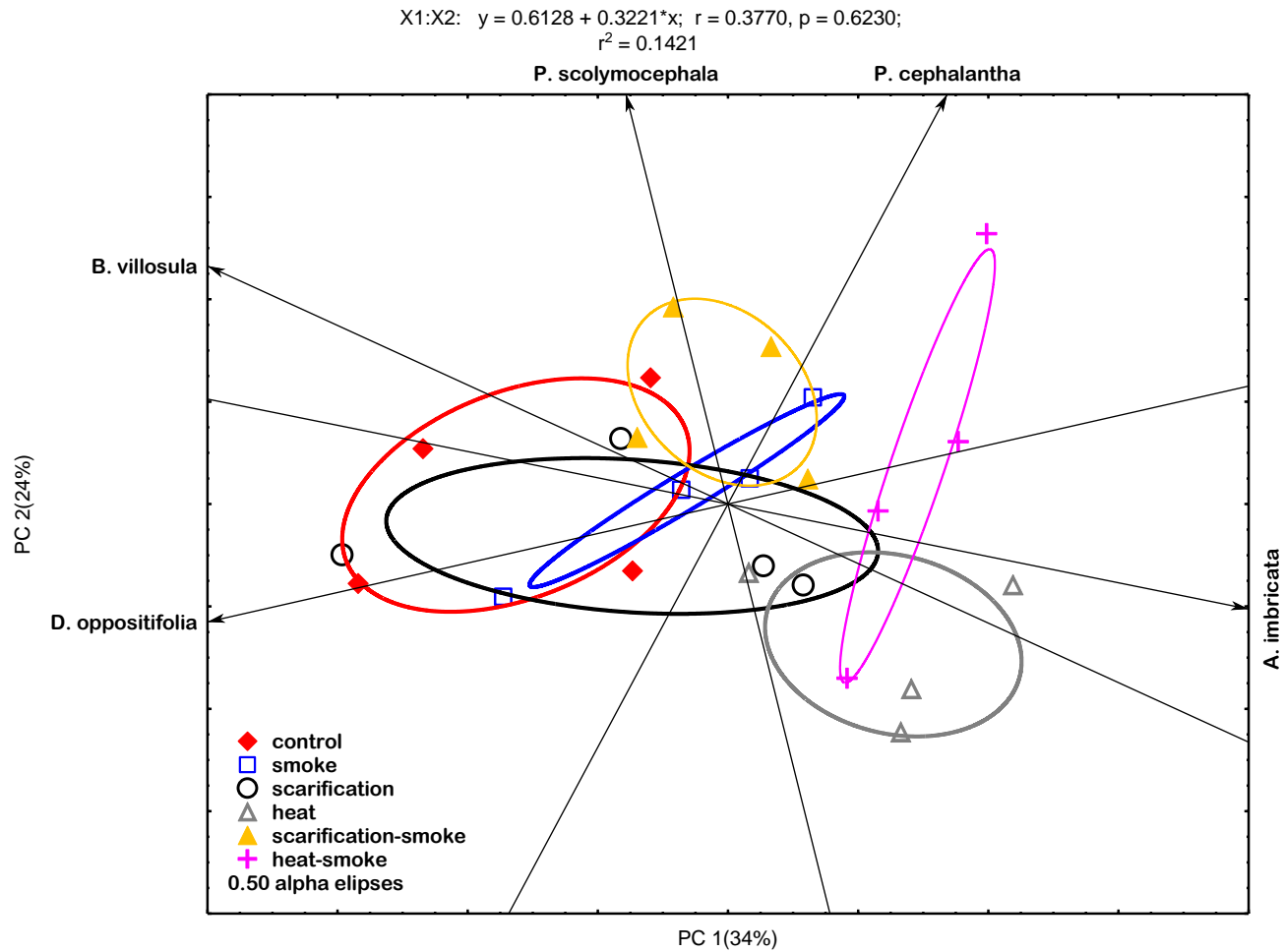
a) Control, smoke, heat and a combination of heat and smoke water pre-germination treatments.

◆ control
● smoke
0.50 alpha ellipses

X1:X2: $y = 1.3773 - 0.0455*x$;
 $r = -0.0430$, $p = 0.9570$; $r^2 = 0.0018$
Senecio elegans



b) Control, smoke water pre-germination treatments.



c) Control, smoke water, heat, scarification, combination of heat and smoke water, a combination of scarification and smoke water pre-germination treatments.

Figure 2.2: Vector overlays on the PCA of the pre-germination treatments, showing seeds of the target CFSF species showing their relationship with the response to pre-germination treatments.

CHAPTER 3: VARIATION IN SEED GERMINATION OF CAPE FLATS SAND FYNBOS SPECIES AFTER GASEOUS SMOKE PRE-GERMINATION TREATMENTS.

ABSTRACT

The Cape Flats Sand Fynbos (CFSF) is a critically endangered vegetation type that is threatened by urban development, agricultural development and invasive alien species. A large-scale restoration project was initiated to re-establish indigenous species following clearing and burning of invasive *Acacia* species. Smoke-derived compounds are known to promote seed germination, but after a burn in Blaauwberg Nature Reserve, sowed seed germination rates recorded were low. Low germination of re-introduced indigenous species could be attributed to a lack of pre-germination treatments prior to sowing.

Thus, to successfully restore the critically endangered Cape Flat Sand Fynbos (CFSF) a better understanding of smoke as a pre-germination cue is required. We conducted a greenhouse experiment to investigate the effectiveness of smoke as a pre-germination treatment in either soil or seeds by mimicking field conditions after a burn. Hence, this study evaluates the effectiveness of three different smoke treatments on the successful germination of a number of typical CFSF plant species, and may contribute to the future success of restoration projects in a critically endangered vegetation type.

The study showed that plant-derived smoke had the ability to enhance seed germination of a number of plant species, and that treatment success was species-specific, with pre-smoked seeds, soil pre-germination treatment and the two combined all capable of significantly boosting germination. It is therefore recommended that seeds be pre-smoked before sowing, especially in the absence of a prescribed burn. Since fynbos plant materials were used in the experiment, further research is needed on the volatile effects of *Acacia saligna* biomass during combustion in the field.

Keywords: Dormancy; Enhanced germination; Plant-derived smoke; Restoration; Smoke-stimulated.

3.1. INTRODUCTION

Port Jackson willow (*Acacia saligna* (Labill.) H.L.Wendl.) has invaded many of the remaining remnants of Cape Flats Sand Fynbos (CFSF) (Rouget et al., 2004; Rebelo et al., 2006) including Blaauwberg Nature Reserve (BBNR). BBNR is located about 25 km north of Cape Town, on the West Coast of the Western Cape, South Africa. Alien trees (mainly *Acacia saligna*) have invaded about 500 ha of CFSF in the BBNR prompting the Biodiversity Management Branch of the City of Cape Town to initiate an ecological restoration research project (Holmes et al., 2008b). Approximately 96 ha of alien vegetation have already been cleared as part of the restoration project. The remaining 400 ha will be cleared and restored based on the findings of this and other research studies within the study area (P. M. Holmes pers. com.). This research area was cleared between September 2012 and April 2013 using two methods, 'Fell and Stack' (with winter burning of stacks) and 'Fell and Burn' (with an early autumn block burn of the felled slash).

However, after removing invasive alien *Acacias* using the Fell and Burn method, regeneration was dominated by *Acacia* recruited from the soil-stored seed bank (Stuart Hall, unpublished) while in the unburnt area (using the Fell and Stack method) germination rates of both indigenous species and *Acacia saligna* were low. The poor germination and low recruitment density of indigenous species poses a challenge for active restoration (Florentine et al., 2011). Previous studies have shown that clearing alone may be insufficient to restore a structurally and functionally viable indigenous community in long-invaded stands, and that additional management strategies are needed (Holmes, 2002; Holmes et al., 2008b; Gaertner et al., 2012; Ruwanza et al., 2013). One such strategy is re-introducing a range of indigenous species of different structural guilds (Holmes et al., 2008a; Cowell, 2013).

Fynbos species are adapted to fire and their seeds respond to physical (i.e. light, temperature, moisture) and/ or germination cues associated with fire (i.e. smoke, heat, chemicals in ashes/ charred wood) (Brown, 1993; van Staden et al., 2000). As was established in the last chapter, smoke treatment enhanced germination of seeds of the CFSF species. However, after a block burn, selected fynbos species were re-introduced in a sowing experiment comprised of sowing fast-growing annuals and species representing the growth form and guild structure (perennial seed mix) of the area. Thus far, germination success has been low and few species from the fynbos perennial seed mix have established (Stuart Hall, unpublished). Moreover, after six months, no resprouting shrub species have established following the sowing, resulting in an under-representation of guild structure and growth form in the field. This may be due to a lack of pre-germination treatments on seeds prior to sowing (which were looked at into detail in Chapter 2), but now will need to look into smoke pre-germination treatment closely. The timing of sowing one month after the prescribed burn could be another possible reason. The persistence of combusted plant-derived smoke impregnated in the soil deteriorates with time, thus affecting the smoke's germination activity or stimulatory effects on seeds (Peart, 1984; Preston and Baldwin, 1999; Ghebrehiwot et al., 2011).

This study evaluates whether germination and establishment in a post-fire environment could be improved by pre-treating CFSF species' seeds with smoke. Fynbos is a Mediterranean type, fire-driven ecosystem and seed germination of both invasive *Acacias* and indigenous fynbos species occurs after fire in conjunction with the winter rainfall (Brown and Botha, 2004). Indigenous uninvaded vegetation regenerates successfully, both structurally and compositionally, with a fire frequency of between 55 and 40 years (Kruger, 1979; van Wilgen and Kruger, 1981). Indirect fire-related germination cues include light and favourable temperature conditions (Bond and van Wilgen, 1996) that are modified because of the burn (increased light, moisture and temperature), removing the litter and taller plants. Favourable post-fire conditions include resource availability and reduced competition and herbivory (Gill, 1981; Keeley, 1991; Whelan, 1995; Tyler, 1996; Bell, 1999).

Germination cues relating to fire include heat shock (Bell et al., 1993), smoke and/or smoke-derived products (De Lange and Boucher, 1990; Keeley and Fotheringham, 1997; Flematti et al., 2004; Light et al., 2010), and combinations of these cues (Brown and Botha, 2004). Smoke triggers seed germination and can stimulate strong promotive responses in many species (Brown, 1993; Dixon et al., 1995; Roche et al., 1998). For restoration purposes, smoke is either applied in gaseous or aqueous form. Smoke applications can either be conducted *in situ* to indigenous habitat or under laboratory or greenhouse conditions. Positive germination response of smoke on seeds of both chaparral (Keeley and Bond, 1997; Keeley and Fotheringham, 1997) and fynbos (De Lange and Boucher, 1990; Brown and Botha, 2004) indicate that germination in seeds of certain species is stimulated by environmental chemical changes (Brown, 1993; Baxter and van Staden, 1994).

Most fynbos species respond positively, with improved germination rates, to fire-related cues such as heat and smoke (Brown, 1993). So far, studies have focused on the extent to which dormancy is broken by fire and smoke (Keeley and Fotheringham, 1997; Brown and Botha, 2004). As seen previously in the pre-germination treatment study (Chapter 2) on CFSF species' seeds, results showed that smoke was an important pre-germination treatment for improving germination in these species. However, little is understood about how seeds respond to smoke as a germination cue and no fynbos restoration studies have compared germination of untreated seeds placed on Smoke-treated soil (i.e., post-fire sowing) versus smoke-treated seeds placed in untreated soil or a combination of both. I used a greenhouse experiment to investigate the effectiveness of smoke pre-germination treatments in either soil or seeds by mimicking field conditions after a burn. This study evaluates the effectiveness of three different smoke treatments on the successful germination of a number of typical CFSF plant species, and may contribute to the future success of restoration projects in a critically endangered vegetation type. Hence, results of this study will provide recommendations for active restoration projects that involve sowing fynbos seed.

3.2. MATERIALS AND METHODS

3.2.1. Species selection and study site

The study was conducted in a greenhouse with a clear corrugated PVC roof in the Forestry Department at Stellenbosch University. The surrounding shade nets in the greenhouse and a fan ensured the daily temperatures fluctuated between 8-25°C, while the roof protected the soil from rain. Study species were chosen based on growth form, regeneration mode, and availability of seeds (Table 1.1). As done in the previous chapter (Chapter 2) seeds were collected, cleaned, and stored in 15% RH and 15° C. The different smoke-treatments were applied to 160 seeds of each species (4 replicates × 40 seeds each) (International Seed Testing Association, 1976). Seed trays (300 × 270 × 100 mm) were lined with a layer of non-woven fabric to retain sand. Dry river-washed sand was used as the first layer of soil (30 mm deep) and the tray was then filled with soil from the BBNR site (70 mm deep). Soil was collected from the mole rat heaps, which are free of *Acacia* leaf litter at BBNR in uncleared, invaded fynbos. This was done to try to exclude *Acacia saligna* seeds from collected soil.

3.2.2. Experimental design

Each tray was divided between two species (each species' seed was allocated 150 mm space), a small-seeded, and a large-seeded species, to accommodate all species within the available space (Figure 3.1). The four pre-germination treatments tested were:

- (i) Control: both untreated seeds and soil (control treatment)
- (ii) Untreated soil with pre-smoked seeds (smoke-treated seeds)
- (iii) Pre-smoked soil and untreated seeds (smoke-treated soil)
- (iv) Pre-smoked seeds and soil (smoke-treated combination)

Dry seeds (placed in replicates of 4×25 per tray spread evenly) soil, a combination of soil and seeds were placed in trays lined with non-woven fabric to retain seeds and/ or soil, then placed in a steel-framed plastic tent. Fynbos biomass was burnt (mixed fresh and dry fynbos plant material) (total worth 25 kg in weight) in a large metal drum. Smoke was then pumped into the tent through a long black plastic pipe (De Lange and Boucher, 1990; Dixon et al., 1995; Brown and Botha, 2004) using a petrol leaf blower. The use of long pipe allowed the smoke to cool before entering the tent. For single treatments, the seeds were placed in trays (300 × 270 × 100 mm) then smoke-treated before sown in soil whereas soil samples in trays (300 × 270 × 100 mm) were smoke-treated before seeds were sown. The combination of dry soil and seeds were pre-smoked in a 10 × 10 × 6 m steel-framed tent, which was sealed with plastic.

Trays were removed after two hours, watered, and then transferred to benches in the greenhouse. Large seeds were buried in rows 2 cm deep into the soil. Small seeds were mixed in with dry sand of which a thin layer was spread evenly over the soil surface. Single treatment trays were moistened to field capacity before planting any seeds; this was done by adding 200 ml of water to each tray. The trays were kept moist using an automated irrigation system that was operational every three days for 3 minutes, providing sufficient water (approximately 5 mm) to keep the soil moisture above wilting capacity. Trays were rotated weekly to account for minor variations in light intensity, temperature, and amount of water in the greenhouse. No fertilisers were added. Seedlings (defined as such when true leaves were observed) were counted weekly, identified, and marked with toothpicks. The experiment ran for over 24 weeks, from April to October 2014.

3.2.3 Statistical analysis

All statistical analyses were performed using the software package STATISTICA© version 12 (StatSoft, Inc.: Tulsa, Oklahoma 2015) with an Alpha (α)-level of 0.05. Using both probability plots and normal expected frequency histograms, data were tested for normality including Levene's test for Homogeneity of Variances. 'Seedling emergence' (as defined below) was used to determine the response of the different species to the different pre-germination treatments.

Seedling emergence was defined as the overall percentage of seeds surviving germination following a pre-germination stimulus. Percentages were used to run a mixed model one-way Analysis of Variance (ANOVA) to test for significant differences in mean seedling emergence among pre-germination treatments. Wherein, in the Variance Estimation and Precision an alternative to ANOVA estimation was provided by restricted maximum likelihood estimation (REML), this was used to generate cumulative plots (pre-germination treatments vs time) per species. Where ANOVAs were significant, I used a post hoc Fisher LSD test to determine significant differences among smoke pre-germination treatments. The results were summarised in a table showing means and standard Errors.

Ordination analysis (Principle component Analysis (PCA)) was used to determine similarity in species' seedling emergence responses to different smoke pre-germination treatments and to determine whether the species response showed patterns in terms of regeneration mode, growth form and seed size. Data were first transformed using $\text{Log}(X+1)$ before running the PCA analysis. The amount of variance (the spread of data values) in the samples was determined using biplots. The species' seed responses to the different pre-germination treatments were plotted according to their total germination percentage. Observations of how certain data points cluster together and whether specific smoke pre-germination treatments are more common among those clusters were then made. Amongst these variables, species with similar responses were grouped next to each other and species with dissimilar responses were dispersed further away from each other.

In this study, the term 'emergence rate' refers to how quickly seedling emergence occurs over given periods and is compared using cumulative germination curves. To show seedling emergence in relation to different smoke pre-germination treatments, I generated cumulative curve plots.

3.3 RESULTS

3.3.1. Seedling emergence in response to smoke treatment

The results of the gaseous smoke pre-germination treatment experiment varied among species. Smoke-treated soil enhanced seed germination in 70% of the species, wherein there was significant differences in 4 out of the 17 species (*Metalasia densa*, *Phyllica cephalantha*, *Serruria fasciflora* and *Thamnochortus punctatus*). Smoke-treated seeds enhanced in germination in 70% of the species, wherein 5 out of the 17 species were significantly different. The combination of smoke-treated seeds and soil enhanced seed germination in 59% of species, wherein there was significant difference in 4 out of the 17 species (*Diosma oppositifolia*, *Phyllica cephalantha*, *Serruria fasciflora* and *Thamnochortus punctatus*).

Species with low germinability in all treatments (<49%) were *Diosma oppositifolia*, *Serruria fasciflora*, and *Thamnochortus punctatus* (Table 3.1). Conversely, *Chrysocoma ciliata*, *Erica mammosa*, *Passerina corymbosa*, *Protea repens*, *Protea scolymocephala*, *Senecio elegans*, *Ursinia anthemoides*, *Wachendorfia multiflora*, and *Watsonia meriana* exhibited relatively high germinability with over 50% germination recorded in all the smoke pre-germination treatments compared to the control treatment (Table 3.1, Figures 3.2, 3.3 and Figure B.1, a-q).

The smoke-treated seeds significantly increased the percentage seedling emergence in *Agathosma imbricata*, *Erica mammosa*, *Erica plumosa*, and *Leucadendron salignum* and *Protea repens* (Table 3.1 and Figures 3.2, 3.3). Smoke-treated soil increased the percentage seedling emergence response in *Metalasia densa*, *Phyllica cephalantha*, *Serruria fasciflora* and *Thamnochortus punctatus* (Table 3.1 and Figures 3.2, 3.3). The smoke-treated soil and the combination of smoke-treated seeds and soil increased germination response in *Phyllica cephalantha*, *Serruria fasciflora*, and *Thamnochortus punctatus* (Table 3.1 and Figures 3.2, 3.3). Only *Diosma oppositifolia* seedlings had a significant positive response to a combination of smoke-treated seeds and soil. Species like *Chrysocoma ciliata*, *Passerina corymbosa*, *Ursinia anthemoides*, *Wachendorfia multiflora*, and *Watsonia meriana* had a significant positive germination response to all smoke treatments as opposed to control (Table 3.1 and Figures 3.2, 3.3).

3.3.2. Seedling emergence rate

Judging by the slope of response the cumulative germination curves indicated that smoke-treated seeds responded more quickly than seeds in smoke-treated soil in the following species: *Erica plumosa* (Figure B.1c), *Erica mammosa* (Figure B.1i) (although the slope of germination was very similar in smoked seeds and smoked soil. The main difference was that the final germination was greater in smoke-treated seeds compared with smoke-treated soil), *Protea repens* (Figure B.1j) and *Passerina corymbosa* (Figure B.1o).

A combination of smoke-treated seeds and soil initiated a faster germination rate response than in smoke-treated seeds alone for *Chrysocoma ciliata* (Figure B.1e), *Diosma oppositifolia* (Figure B.1f), *Senecio elegans* (Figure B.1h), *Phyllica cephalantha* (Figure B.1m), *Thamnochortus punctatus* (Figure B.1p) and *Ursinia anthemoides* (Figure B.1q).

A faster germination rate response was recorded in the smoke-treated soil than in the combination of seeds and soil treatment in *Metalasia densa* (Figure B.1a), *Serruria fasciflora* (Figure B.1d), *Protea scolymocephala* (Figure B.1g), *Agathosma imbricata* (Figure B.1k), *Leucadendron salignum* (Figure B.1l), and *Wachendorfia multiflora* (Figure B.1n). Germination rates in *Watsonia meriana*, however, did not differ among smoke pre-germination treatments (Figure B.1b).

Preliminary descriptive analysis using Principal Component Analysis (PCA) shows some pattern for growth form that showed a clustering of shrubs and geophytes around a combination of smoke-treated seeds and soil (Figure 3.3 a). Regeneration mode showed a pattern of clustering of reseeders and resprouters around the combination of smoke-treated seeds and soil (Figure 1.3 b). Seed size showed a clustering of small, medium and big sized seeds around the combination of smoke-treated seeds and soil too (Figure 3.3c). The responses were species-specific.

3.4. DISCUSSION

3.4.1. Seedling emergence and rate

This research evaluated treatments in stimulating seed germination using gaseous smoke for ecological restoration applications in Cape Flats Sand fynbos (CFSF) vegetation. This study was initiated due to the poor germination response after seeds were sown in the BBNR field following clearance of invasive alien vegetation and an autumn burn. Germination of fynbos seeds can be enhanced by smoke and/or heat (Brown, 1993; Read et al., 2000). All species except for two showed enhanced germination after smoke-treatment. Similarly, Bargmann et al. (2014) found that smoke was a more effective germination cue across all functional groups than ashes were in northern heathland ecosystems. Smoke-stimulated germination is found in a wide range of families from both fire-prone (De Lange and Boucher, 1990; Brown, 1993) and fire-resistant environments (Pierce et al., 1995; Keeley and Fotheringham, 2000) which enables those species that lack heat-stimulated germination to respond to chemical products of combusted biomass. The combustion chemical products that enhance germination are transferred to seeds in gaseous or aqueous form and this may occur directly from smoke or be secondarily transferred from soil particles to seeds (van Staden et al., 2000). The fact that smoke was found to be more effective in the combination of soil and smoke-treated seeds, is in consent with a study by Keeley and Fotheringham, (1998).

This study showed that although smoke is an important germination cue for most CFSF species and guilds, the different pre-germination treatments types (smoke-treated seed, soil and combined combination of smoke-treated seeds and soil) differed in their effect on germinability among species. In general, species response to the different pre-germination treatments showed some patterns associated with growth forms, regeneration mode or seed size. Species with the same growth form or regeneration mode varied in their response to the different pre-germination treatments suggesting that responses to pre-germination treatments were species-specific. Germination in both large-seeded and small-seeded species was enhanced significantly by smoke pre-germination treatments. Likewise, Dixon et al., (1995) found that both large- and small-seeded plant species responded equally positively to different smoke pre-germination treatments in Western Australian. Furthermore, studies by De Lange and Boucher, (1990), Brown, (1993) and Dixon et al., (1995) recorded smoke-stimulated germination in serotinous species, namely *Actinostrobus acuminatus* (West Australian member of Cupressaceae) and *Protea repens* (South African member of the Proteaceae).

In conclusion, this study shows that plant-derived gaseous smoke pre-germination treatments enhance seed germination in numerous CFSF species. The success of the type of gaseous smoke pre-germination treatment applied, however, was species-specific. Seeds sown in one year may germinate in the following year; however, results of this study are of considerable importance as using smoke pre-germination treatments to improve germination may be critical to successful restoration programmes that depend on seed sowing and prescribed burns. This experiment might have underestimated final seedling emergence in the field. Other factors could have influenced seed germination and emergence such as temperature and light.

3.4.2. Recommendations for restoration

Results from the greenhouse germination study show that the majority of CFSF species require smoke-treated soil, seed, or a combination of both to enhance germination and should be included in restoration protocol. Seeds should be pre-smoked in a tent and, if restoration efforts include controlled burns, smoke treatment should be applied to the seeds. This is because, in many cases, sowing may be between one month and five months after a fire (for a November/December burn) and smoke chemicals in the soil may have dissipated over time, especially with interim rainfall. It is important for seeds to be sown in the field before the winter rainfall (from May to August) to allow species sufficient time to establish in moist soil conditions. Smoke effects may be seasonally operative (Enright and Lamont, 1989; Auld and O'Connell, 1991; van Wilgen et al., 1992; Bond and van Wilgen, 1996), with the stimulatory effects declining in late autumn (De Lange and Boucher, 1993), thus timing of the seed smoke pre-germination treatment could be important (Ghebrehiwot et al., 2011). Based on this study, the high germination rates of *W. meriana*, *C. ciliata*, *E. mammosa*, *P. scolymocephala*, *S. elegans*, *P. corymbosa* and *U. anthemoides* in control treatments indicates that these species should be targeted for active restoration of the CFSF if smoke pre-germination treatment is not an option. To ensure a structurally diverse and fully functional fynbos, the under-represented guilds should be targeted for re-introduction.

3.4.3. Research need

In this study, fynbos species were used as combustion biomass. However, the actual field combustion biomass in areas where *Acacias* has been removed. It is usually a combination of fynbos and *Acacia saligna* (Jones et al., 1963). It may be worth investigating volatile chemical compounds of burnt *Acacia saligna* as their effects on seed germination of indigenous fynbos species may differ from that of fynbos material.

It may also be important to run a field experiment parallel with a greenhouse study to complement the findings of this study. Doing this would eliminate confounding factors such as interactions with other germination cues, and time since fire and season that could contribute to the response or lack of response of germination in fynbos species. To summarise, the effectiveness of the three smoke pre-germination treatments used in this study is species-specific, hence both seeds and soil should be smoke treated.

REFERENCES

- Auld, T.D., O'Connell, M.A., 1991. Predicting patterns of post-fire germination in 35 eastern Australian Fabaceae. *Australian Journal of Ecology* 16, 53-70.
- Bargmann, T., Maren, I.E., Vandvik, V., 2014. Life after fire: smoke and ash as germination cues in ericads, herbs, and graminoids of Northern heathlands. *Applied Vegetation Science* 17, 670-679.
- Baxter, B.J.M., van Staden, J., 1994. Plant-derived smoke: an effective seed pre-treatment. *Plant Growth Regulation* 14, 279-282.
- Bell, D.T., 1999. Turner review No. 1: the process of germination in Australian species. *Australian Journal of Botany* 47, 475-517.
- Bell, D.T., Plummer, J.A., Taylor, S.K., 1993. Seed germination ecology in South-western Australia. *The Botanical Review* 59, 24-73.
- Bond, W.J., van Wilgen, B.W., 1996. *Fire and plants*. 1st edition. Chapman and Hall, London.
- Brown, N.A.C., 1993. Promotion of fynbos seeds by plant-derived smoke. *New Phytologist* 123, 575-583.
- 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, 559-581.
- Cowell, C., 2013. Investigating the most favourable seed establishment methods of restoring sand fynbos on old fields. Unpublished Master of Technology thesis, Cape Peninsula University of Technology.
- De Lange, J.H., Boucher, C., 1990. Autecological studies on *Audouinia capitata* (Bruniaceae). 1. Plant derived smoke as a seed germination cue. *South African Journal of Botany* 55, 700-703.
- Dixon, K.W., Roche, S., Pate, J.S., 1995. The promotive effect of smoke derived from burnt native vegetation on seed germination of western Australian plants. *Oecologia* 101, 185-192.

- Enright, N.J., Lamont, B.B., 1989. Seed banks, fire season, safe sites and seedling recruitment in five co-occurring *Banksia* species. *Journal of Ecology* 77, 1111-22.
- Florentine, S.K., Graz, F.P.; Ambrose, G., O'Brien, L., 2011. The current status of different age, direct-seeded revegetation sites in an agricultural landscape in the Burrumbeet region, Victoria, Australia. *Land Degradation and Development* 1-9.
- Gaertner, M., Holmes, P.M., Richardson, D.M., 2012. Biological invasions, resilience and restoration. In: van Andel, J., Aronson, J. (Eds.). *Restoration Ecology: the new frontier*, 2nd edition. Wiley-Blackwell, Oxford, pp. 265-280.
- Gill, A.M., 1981. Adaptive responses of Australian vascular plant species to fire. In: Gill, A.M., Groves, R., Noble, I. (Eds.), *Fire and the Australian biota*. Australian Academy of Science 243-271.
- Holmes P.M., 2002. Depth distribution and composition of seed-banks in alien-invaded and uninvaded fynbos vegetation. *Austral Ecology* 27, 110-120.
- Holmes, P., Wood, J., Dorse, C., Biodiversity Management Branch, 2008b. City of Cape Town biodiversity report. pp. 56-62.
- Holmes, P.M., Esler, K.J., Richardson, D.M., Witkowski, E.T.F., 2008a. Guidelines for improved management of riparian zones invaded by alien plants in South Africa. *South African Journal of Botany* 74, 538-552.
- International Seed Testing Association 1976: *International Rules for seed testing. Rules and annexes*. International Seed Testing Association, *Seed Science and Technology* 4, 3–177.
- Jones, R.M., Roux, E.R., Warren, J.M., 1963. Studies in the autecology of Australian acacias in South Africa. 4. The production of toxic substances by *Acacia cyclops* and *Acacia cyanophylla* and their possible ecological significance. *South African Journal of Science* 59, 295-296.
- Keeley, J.E., 1991. Seed germination and life history syndromes in the Californian chaparral. *Botanical Review* 57, 81-116.
- Keeley, J.E., Bond, W.J., 1997. Convergent seed germination in South African fynbos and Californian chaparral. *Plant Ecology* 133, 153-167.

- Keeley, J.E., Fotheringham, C.J., 1997. Trace gas emissions and smoke-induced seed germination. *Science* 276, 1248-1250.
- Keeley, J.E., Fotheringham, C.J., 2000. Chapter 13: Role of fire in regeneration from seed. In: Fenner, M. (Ed.), *Seeds: The ecology of Regeneration in Plant Communities*, 2nd edition. CAB International. pp. 311-330.
- Kruger, F.J., 1979. South African heathlands. In: Specht, R.L. (Ed.) *Ecosystems of the world*. Vol. 9 Heathlands and related shrublands. Amsterdam, Elsevier 19-80.
- Pierce, S.M., Esler, K., Cowling, R.M., 1995. Smoke-induced germination of succulents (*Mesembryanthemaceae*) from fire prone and fire-free habitats in South Africa. *Oecologia* 102, 520-522.
- Read, T.R., Bellairs, S.M., Mulligan, D.R., Lamb, D., 2000. Smoke and heat effects on soil seed bank germination for the re-establishment of a native forest community in New South Wales. *Austral Ecology* 25, 48-57.
- Rebelo, A.G., Boucher, C., Helme, N.A., Mucina, L., Rutherford, M.C., 2006. Fynbos biome. In: Mucina, L, Rutherford M.C., (Eds.). *The Vegetation of South Africa, Lesotho and Swaziland: Strelitzia*, 19, pp. 52–219.
- Roche, S., Dixon, K.W., Pate, J.S., 1998. For everything a season: smoke-induced seed germination and seedling recruitment in a western Australian *Banksia* woodland. *Australian Journal of Ecology* 23, 111-120.
- Rouget, M., Reyers, B., Jonas, Z., Desmet, P., Driver, A., Maze, K., Egoh, B., Cowling, R.M., 2004. South African National Spatial Biodiversity Assessment 2004: Technical report. Volume 1: Terrestrial component. South African National Biodiversity Institute, Pretoria.
<http://www.bcb.uwc.ac.za/pssa/articles/includes/NSBA%20Vol%201%20Terrestrial%20Component%20Draft%20Oct%2004.pdf>
- 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, 132–141.

- StatSoft Inc., 2015. STATISTICA (Data Analysis Software System), version 12. www.statsoft.com. Tulsa, Oklahoma.
- Tyler, C.M., 1996. Relative importance of factors contributing to post fire seedling establishment in maritime chaparral. *Ecology* 77, 2182-2195.
- van Staden, J., Brown, N.A.C., Jager, A.K., Johnson, T.A., 2000. Smoke as a germination cue. *Plant Species Biology* 15, 167-178.
- van Wilgen, B.W., Bond, W.J., Richardson, D.M., 1992. Ecosystem management. In: Cowling, R.M. (Ed.). *The Ecology of Fynbos: Nutrients, Fire and Diversity*. Oxford University Press, Cape Town. pp. 345-371.
- van Wilgen, B.W., Kruger, F.J., 1981. Observations on the effects of fire in Mountain Fynbos at Zacharias-hoek, Paarl. *South African Journal of Botany* 47, 195-212.
- Whelan, R.J., 1995. *The Ecology of fire*. Cambridge University Press, Cambridge.

Table 3.1: Effects of different smoke pre-germination treatments on percentage germination in 17 target indigenous fynbos species tested under greenhouse conditions. Data are means \pm SE, $n=4 \times 40$ seeds and results of 1-way ANOVA arcsine transformed percentage are shown (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS is not significant $P > 0.05$). Superscript letters show where the significant differences lie within species between the pre-germination treatments after Fisher LSD post hoc test. Treatment with the highest germination percentage for each species is indicated in bold.

Species name	Control	Smoke-treated soil	Smoke-treated seeds	Combination of smoke-treated seeds & soil	ANOVA= $F_{(3,12)}$
<i>Agathosma imbricata</i>	20.63 \pm 3.59 ^c	38.13 \pm 5.98 ^b	80.00 \pm 4.21 ^a	54.38 \pm 7.32 ^b	F(3, 12)=21.250 ^{***}
<i>Chrysocoma ciliata</i>	56.25 \pm 2.17 ^b	82.50 \pm 8.10 ^a	90.00 \pm 3.54 ^a	81.88 \pm 4.83 ^a	F(3, 12)=8.190 ^{***}
<i>Diosma oppositifolia</i>	5.63 \pm 0.63 ^c	15.00 \pm 2.70 ^{ab}	11.88 \pm 3.44 ^{cb}	21.25 \pm 1.25 ^a	F(3, 12)=8.025 ^{***}
<i>Erica mammosa</i>	40.00 \pm 6.77 ^c	53.75 \pm 10.43 ^{cb}	84.38 \pm 5.04 ^a	71.88 \pm 13.40 ^{ab}	F(3, 12)=4.261 [*]
<i>Erica plumosa</i>	11.25 \pm 2.17 ^c	6.88 \pm 1.88 ^c	61.88 \pm 3.87 ^a	36.88 \pm 4.83 ^b	F(3, 12)=55.852 ^{***}
<i>Leucadendron salignum</i>	11.88 \pm 0.63 ^c	59.38 \pm 7.80 ^{ab}	69.38 \pm 7.10 ^a	46.25 \pm 9.16 ^b	F(3, 12)=12.879 ^{***}
<i>Metalasia densa</i>	16.25 \pm 2.39 ^c	74.38 \pm 7.46 ^a	48.75 \pm 5.25 ^b	48.75 \pm 12.84 ^{ab}	F(3, 12)=9.448 ^{***}
<i>Passerina corymbosa</i>	38.75 \pm 8.57 ^b	76.25 \pm 8.07 ^a	70.63 \pm 4.72 ^a	78.13 \pm 6.95 ^a	F(3, 12)=6.478 ^{**}
<i>Phylica cephalantha</i>	19.38 \pm 0.63 ^b	50.63 \pm 6.88 ^a	20.63 \pm 1.88 ^b	44.38 \pm 3.44 ^a	F(3, 12)=16.430 ^{***}
<i>Protea repens</i>	10.63 \pm 3.29 ^c	50.63 \pm 8.19 ^b	93.13 \pm 2.77 ^a	62.50 \pm 10.10 ^b	F(3, 12)=24.840 ^{***}
<i>Protea scolymocephala</i>	45.00 \pm 3.68	55.63 \pm 4.13	56.88 \pm 7.32	65.63 \pm 7.32	F(3, 12)=2.077 ^{NS}
<i>Senecio elegans</i>	74.38 \pm 8.98	79.38 \pm 8.68	65.63 \pm 5.34	83.13 \pm 12.80	F(3, 12)=0.658 ^{NS}
<i>Serruria fasciflora</i>	14.38 \pm 1.88 ^b	36.88 \pm 7.39 ^a	21.25 \pm 2.39 ^{ab}	36.25 \pm 6.65 ^a	F(3, 12)=4.631 [*]
<i>Thamnochortus punctatus</i>	20.00 \pm 5.86 ^{cb}	40.63 \pm 6.80 ^a	7.50 \pm 5.95 ^c	39.38 \pm 7.93 ^{ab}	F(3, 12)=5.723 ^{**}
<i>Ursinia anthemoides</i>	35.63 \pm 4.49 ^b	71.88 \pm 10.96 ^a	83.75 \pm 2.98 ^a	78.13 \pm 13.78 ^a	F(3, 12)=5.551 ^{**}
<i>Wachendorfia multiflora</i>	24.38 \pm 3.29 ^b	74.38 \pm 6.80 ^a	55.00 \pm 6.85 ^a	68.75 \pm 12.48 ^a	F(3, 12)=7.707 ^{***}
<i>Watsonia meriana</i>	36.88 \pm 2.77 ^b	84.38 \pm 7.02 ^a	73.13 \pm 4.72 ^a	88.13 \pm 5.14 ^a	F(3, 12)=20.690 ^{***}

Figures

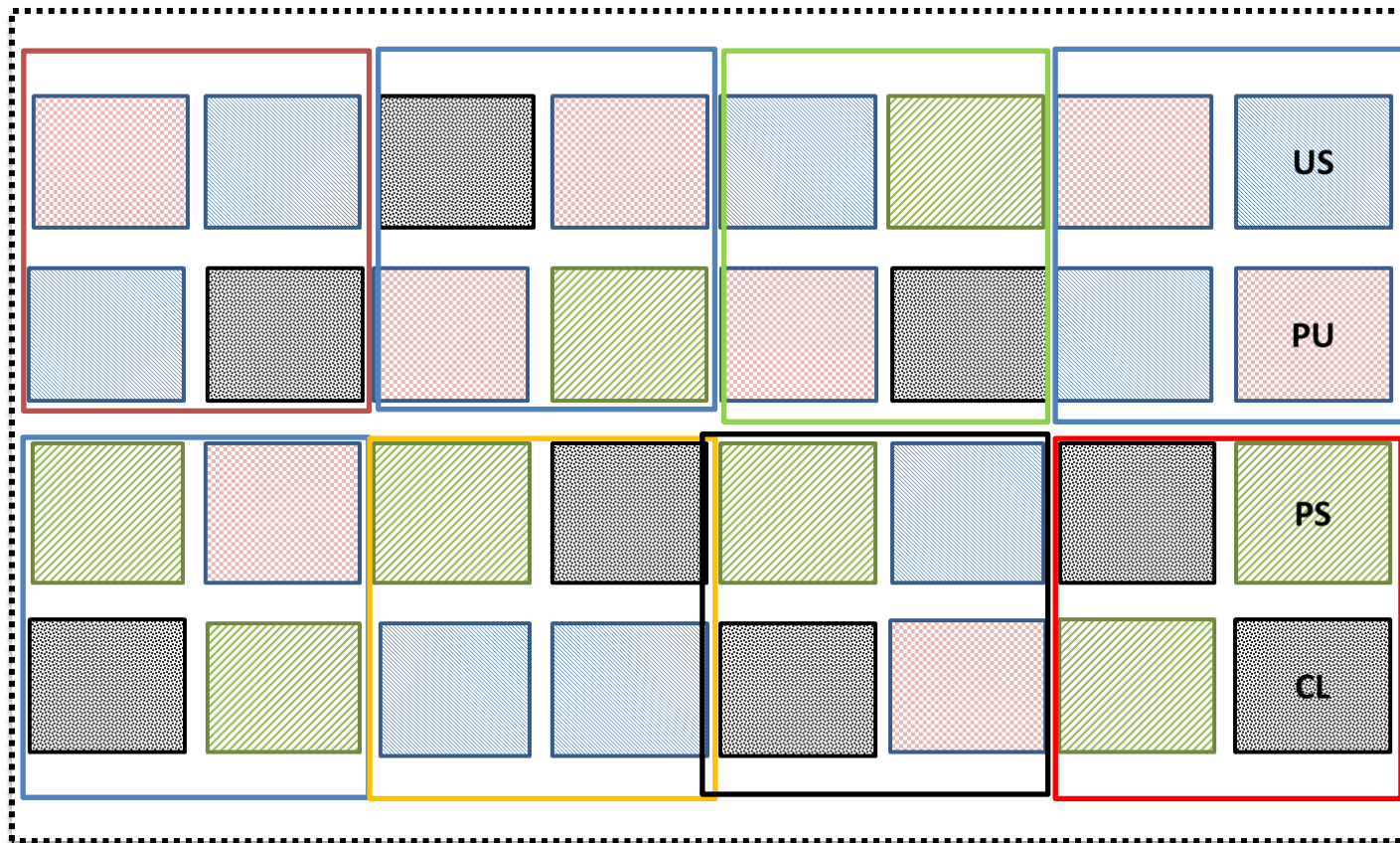


Figure 3.1: Typical example of the randomized experimental design showing a greenhouse set-up of 4 replicates of 40 seeds each for the four pre-germination treatments including pre-germination treatments. Main trays were divided among 4 pre-germination treatments consisting of untreated seeds (US) on pre-smoked soil, smoke-treated seeds(PU) on untreated soil, pre-smoked seeds and soil (PS and control (CL)). The pre-germination treatments were used to enhance seed germination on 17 target species.

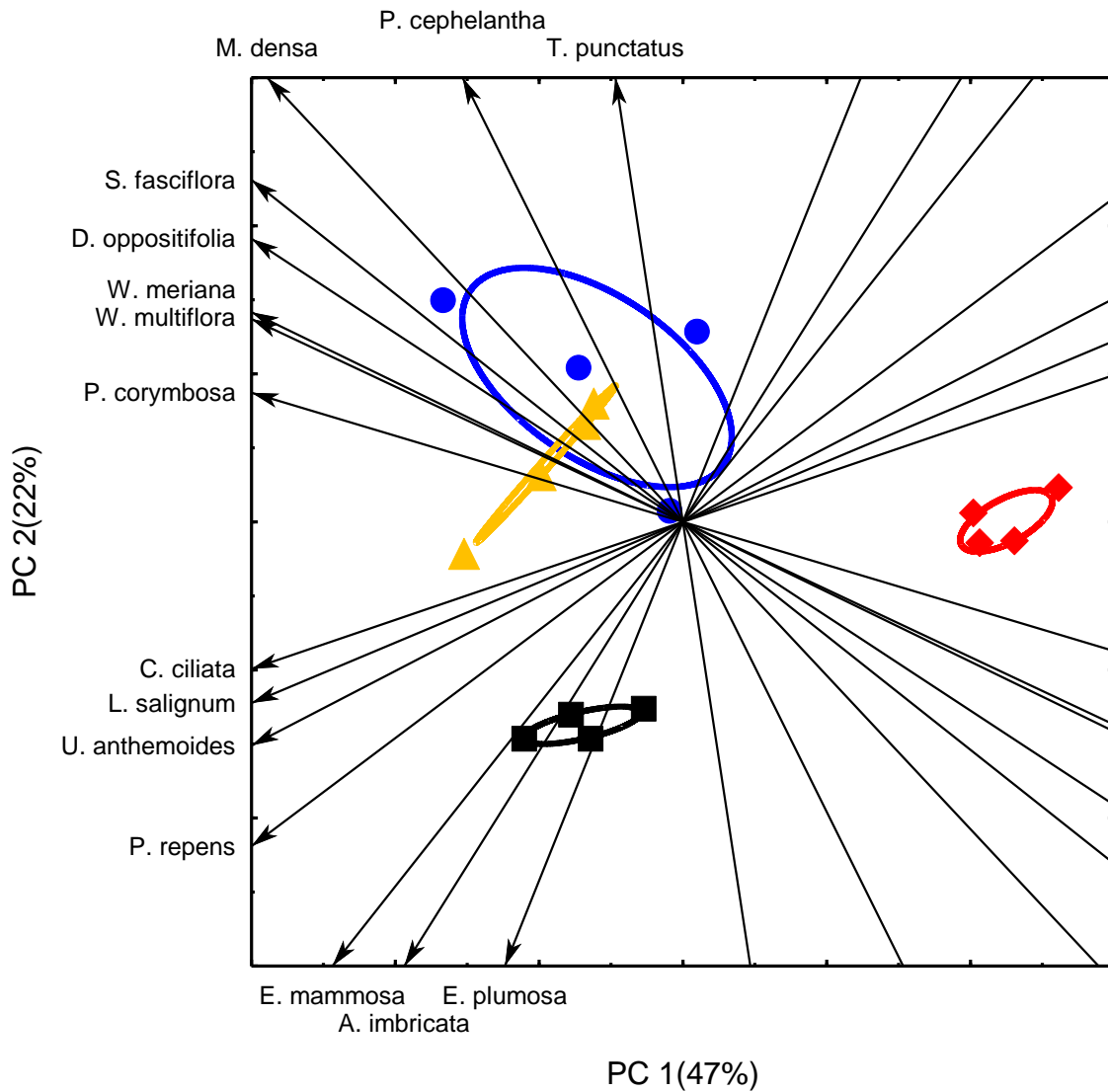
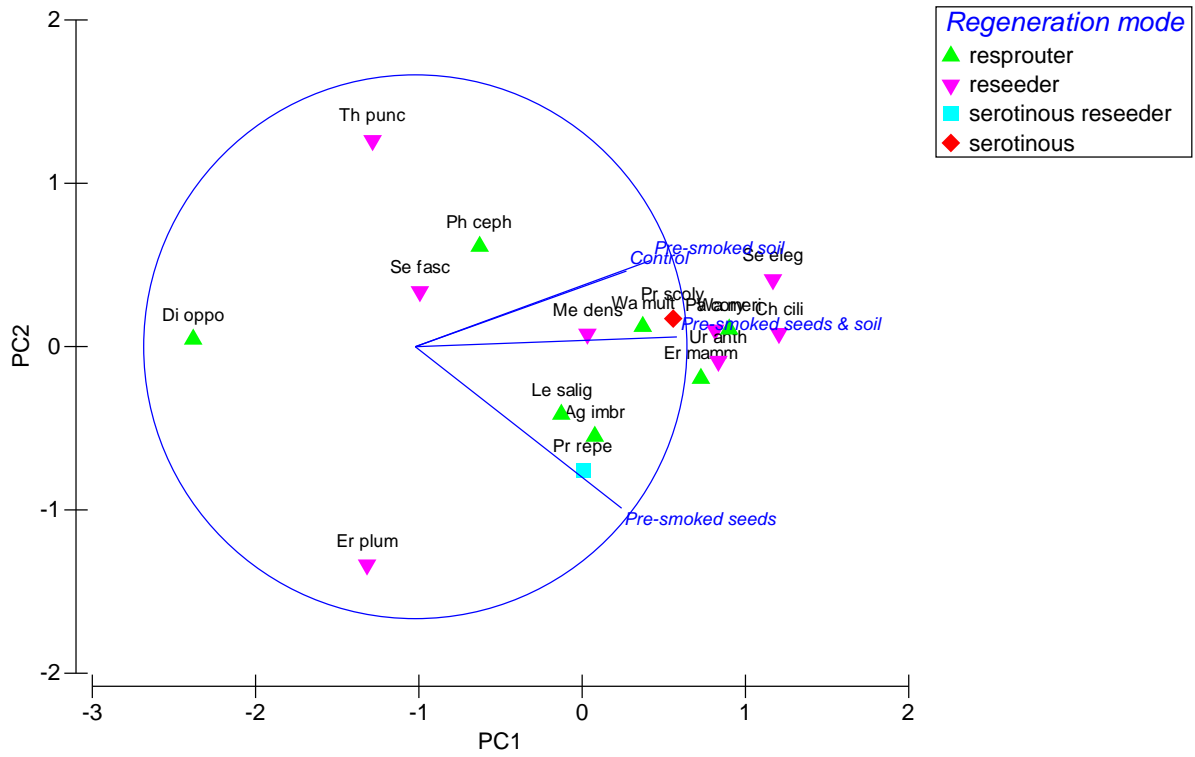
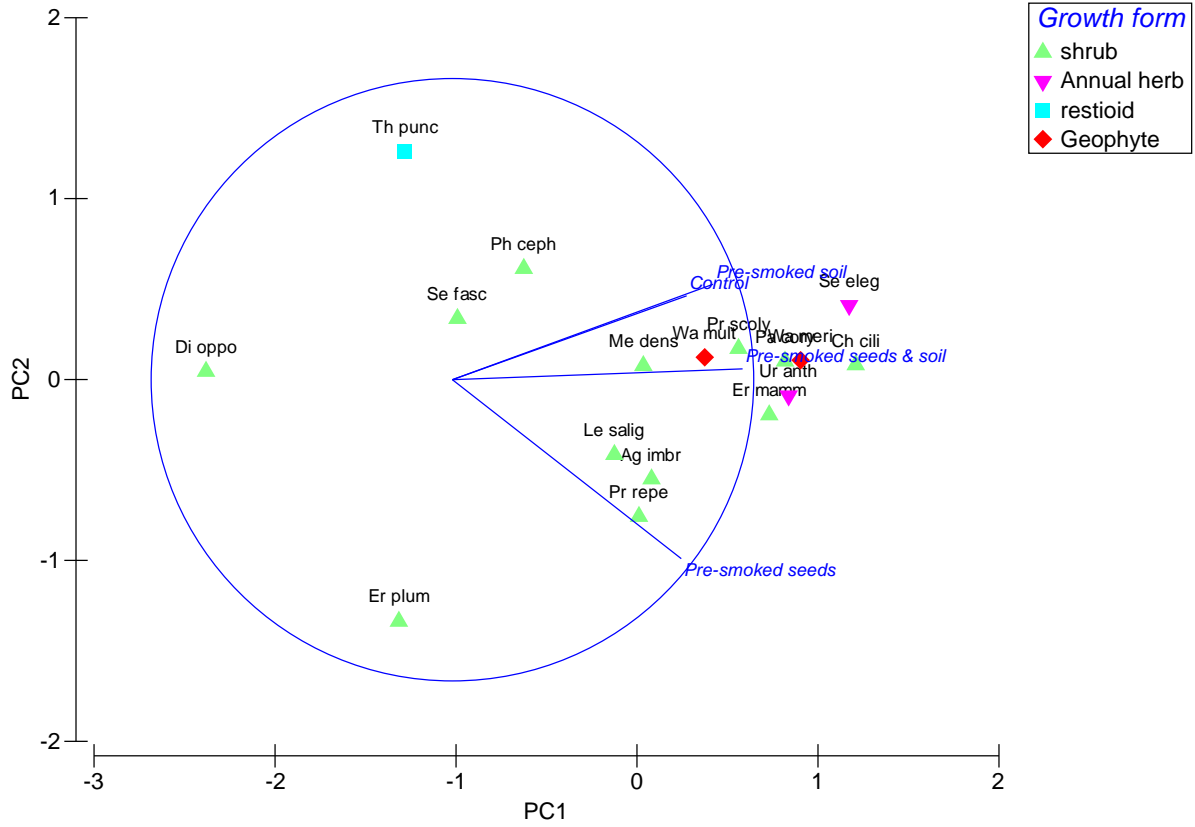


Figure 3.2: Vector overlays on the Principal Component Analysis (PCA) of the smoke pre-germination treatments, showing clusters for germination response of seeds of the Cape Flats Sand Fynbos (CFSF) and their relationship with the plant-derived smoke (gaseous) pre-germination treatments. The key symbols representing the explanatory variables are shown as follows: (♦) control, (■) smoke treated-soil, (●) smoke-treated seeds and (▲) a combination of smoke-treated seeds and soil.



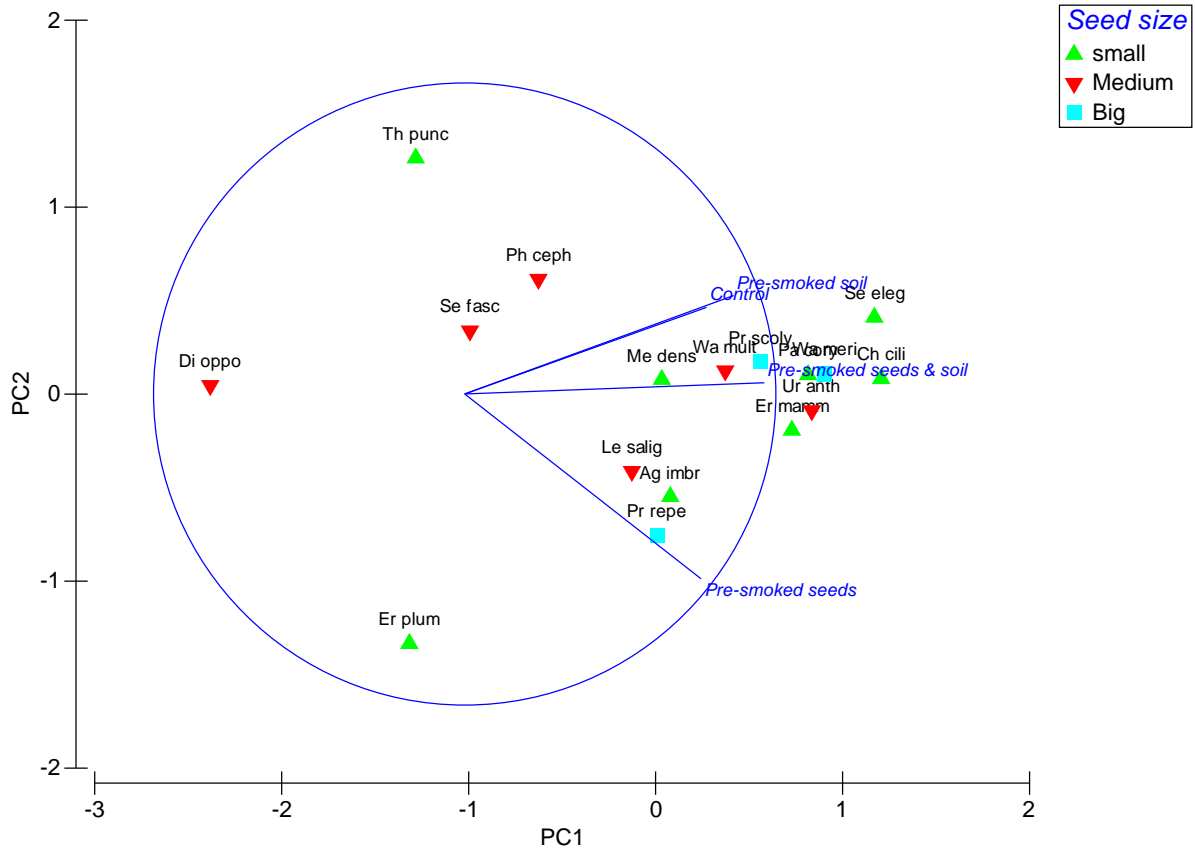


Figure 3.3: Vector overlays on the Principal Component Analysis (PCA) of the greenhouse smoke pre-germination treatments, showing clusters for germination response of seeds of the Cape Flats Sand Fynbos (CFSF) species and the variable patterns they group in terms of a) Growth form b) Regeneration mode and c) Seed size.

CHAPTER 4: CAPE FLATS SAND FYNBOS SPECIES GERMINANTS AND SEEDLINGS' TOLERANCE TO DESICCATION: IMPLICATIONS FOR BLAAUWBERG NATURE RESERVE LARGE-SCALE RESTORATION

ABSTRACT

Blaauwberg Nature Reserve harbours some of the last remaining patches of a critically endangered vegetation type- the Cape Flats Sand Fynbos. This vegetation type is threatened by invasion of Australia *Acacia* species, mainly *Acacia saligna*. Efforts are being made to restore biodiversity back to this vegetation type. These efforts require knowledge on several fronts, including an understanding of constraints to native plant recruitment.

Soil moisture is a limiting factor for seed germination and seedling survival. Fynbos is a nutrient-poor ecosystem adapted to seasonal water stress. Yet, with competition by *Acacia* species drought/ desiccation is suspected for low fynbos recovery (seed germination and seedling emergence) after alien clearing.

Hence, selections of Cape Flats Sand Fynbos species were tested for desiccation tolerance of both germinants and seedlings. Using controlled Low Temperature Germination Growth Chambers (Labcon, Model: LTGC-M-70, South Africa) (with alternating temperatures of 10° C /20° C photoperiod of 10h L/14h D), research was done on the effects of drought/desiccation on seed germinants and seedling emergence. In parallel, evaluation was done for drought/desiccation tolerance of seedlings of the target species in a greenhouse experiment.

Repeated measures ANOVA and Principal Component Analysis revealed that almost all species germinated and they were all reasonably tolerant to drought/desiccation treatment.

Most of the species were desiccation tolerant although a few were sensitive to the drought/desiccation treatments. Drought/desiccation is unlikely to be the cause of limited seed germination and emergence in the field. Mortality of the germinants and seedlings in the field might be due to other constraints or a combination thereof (i.e. granivory, soil chemical properties, increased nutrient-levels, and soil microbial communities).

Keywords: Desiccation tolerance; Germinants; Moisture

4.1. INTRODUCTION

Cape Flats Sand Fynbos (CFSF) is a critically endangered vegetation type in the Cape Floristic Region with only 14% of its historical area remaining (Rebelo et al., 2011). It is rich in endemic plant species (16) as well as species that are classified as threatened on the IUCN Red List (108, including 5 extinct/ extinct in the wild species) (Rebelo et al., 2011). The main threat to this vegetation type is habitat destruction, mostly through the urban expansion of Cape Town, but also through agricultural activities. Secondly, invasive alien species such as Port Jackson willow (*Acacia saligna* (Labill.) H.L. Wendl.) and to a lesser extent Coastal Tea Tree (*Leptospermum laevigatum* (Gaertn.) F. Muell.), which have invaded most remaining areas of this vegetation type (Mehta, 2000; Rebelo et al., 2011). Invasive alien species use large quantities of water, thus changing the level of the ground water table (Rowntree, 1991). The Biodiversity Management Branch of the City of Cape Town initiated a large-scale restoration programme at Blaauwberg Nature Reserve (BBNR), located on the West Coast of South Africa, 25 km north of Cape Town city centre. However, attempts to restore alien-invaded Sand Fynbos are so far of limited success due to low germination and/or seedling establishment rates of sown seeds (Cowell, 2013; Stuart Hall, unpublished). The low establishment rate of indigenous species could be due to either inappropriate management practices e.g. omission of appropriate pre-germination treatments, or ecological factors such as competition by fast growing *Acacia* seedlings for light, lack of moisture (seasonal drought) and desiccation of seeds and seedlings before or after germination.

It has been shown that soil moisture is a limiting factor for seed germination and seedling survival. Desiccation tolerance is a strategy that plants have developed to cope with low soil moisture levels. Desiccation is defined as the ability of a living structure to survive drying with low (<50%) relative humidity and maintain low intracellular water concentrations (Alpert, 2005). A desiccation tolerance response hence means that species are decreasing their water potential during drought. The ability of a plant to avoid or tolerate desiccation depends on its response to water stress via stomatal conductance (Turner, 1986). This includes a range of anatomical, morphological, and physiological features that determine water usage patterns (Alpert, 2005). The features for drought tolerance include high leaf longevity, deep rooting depth, sclerophylly, and small leaf size.

Lack of moisture is often regarded as the major cause of seedling mortality in Mediterranean regions (Mack, 1976; Lamont et al., 1989). Thomas and Davis (1989) concluded that the ability of seedlings to survive desiccation was of much greater importance than initial seedling numbers. Rates of seedling emergence and/or the rates of injury to seedling growth will depend on the underlying environmental stress (i.e. desiccation). The relative tolerance to water insufficiency at any of these stages (i.e., germination, emergence, and seedling growth) will affect seedling mortality rates. It has for example been shown by Mustart et al. (2012) that germinating seeds of Proteaceae are extremely tolerant to desiccation, and that this tolerance is lost upon emergence.

In comparison with other Mediterranean-type ecosystems, fynbos is adapted to nutrient-poor soils and has lower levels of water stress (Stock et al., 1992), which means that water is generally not regarded as a limiting factor in the fynbos. Invasion of fynbos ecosystems by Australian *Acacias*, as well as climate change are, however, major concerns about plant water relations. This is of concern given that future climate change predictions for the Western Cape show the probability of hotter and drier conditions (Midgley et al., 2002). The expected declines in rainfall may drive some indigenous plant species beyond their drought thresholds, causing extensive mortality of more vulnerable species (Midgley et al., 2005).

Rapid seedling emergence is considered advantageous under certain situations, and has been shown to be of great benefit for seedling survival and growth (Jones et al., 1997). Rapid seedling emergence after fire includes the advantages of an increased availability of resources and reduced competition (Whelan, 1995; Bell, 1999). Fynbos is a fire-prone vegetation type (Smith et al., 1992; van Wilgen and Forsyth 1992; Rebelo et al., 2006), with most of its indigenous species being adapted to fire. Benefits of rapid seedling emergence are lost to fynbos seedlings under *Acacia* invasion. Invasive *Acacia* species are also fire-adapted but at the same time fast growing and tend to out-compete slower growing fynbos species (Richardson and van Wilgen, 2004). In invaded ecosystems, water depletion is considered one of the most significant impacts of Australian *Acacia* species (Le Maître, 2004). The increased water use is likely a result of larger above-ground biomass in *Acacia* stands compared to indigenous vegetation. *Acacia* seedlings tend to develop roots 1.5 to 4-fold longer than co-occurring indigenous species, which penetrate deeper into the soil profile (Witkowski, 1991; Morris et al., 2011). Root development usually occurs at a faster rate than for indigenous species (Musil, 1993; Peperkorn et al., 2005), providing *Acacia* seedlings with a competitive advantage to access water, especially during drought periods, over indigenous species (Roche et al., 1994). Another desiccation tolerance trait of *Acacia saligna* is the development of sclerophyllous phyllodes to replace leaves (Pasquet-Kok et al., 2010).

It has been stated that the success of fynbos restoration after alien clearance depends largely on seedling establishment, although a significant amount of seed production and germination is also crucial (Cowling et al., 1997). According to Cowling et al. (1997), seedling recruitment is a function of seedling germination, emergence, establishment, and survival following growth to maturity. Midgley (1988) and van Hensbergen et al. (1992) pointed out that seedling mortality was in general lower in mountain fynbos than in lowland fynbos (Mustart and Cowling, 1993a; Maze and Bond, 1996). However, little is known about the cause of this difference in mortality. In order to gain better understanding about the survival patterns of seedlings, it is necessary to assess the relative importance of moisture related causes of mortality in seedlings.

Several studies have explored seedling desiccation tolerance. The effect of seedling water stress on two sand plain *Banksia* species was investigated in Australia to test their different abilities to tolerate drought, wherein the two *Banksia* species displayed seedling desiccation responses consistent with their current habitat preferences (Groom, 2002). A desiccation study was performed on seedlings of 15 species of British plants, and it was found that all species from drier habitats established better in drier soils than wetland species (Evans and Etherington, 1991). In a study in South African fynbos, Agenbag (2006) and Esler et al. (2015) found that species with high relative growth rates, such as the dominant broad-leaved proteoids, were more sensitive to desiccation than slow growing needle-leaved species of lower vegetation layers. Mustart et al. (2012) found highly individualistic responses to desiccation in seedlings of 23 proteoid species from different habitats.

In this study, laboratory and greenhouse experiments were employed with the aim of exploring desiccation tolerance of seeds as well as seedlings of different plant types in the fynbos. Specifically I sought answers to the following **key questions**: (1) Are seeds of the CFSF species intolerant to desiccation to such extent that it affects their germination and seedling establishment? (2) Is there a correlation between moisture regime (i.e. desiccation) and seed germination or seedling survival? (3) Is there a correlation between tolerance to desiccation treatment, regeneration mode, growth form and seed size?

Although there have been studies performed on plants' sensitivity to desiccation, there are still gaps with regard to lack of moisture as a limiting factor to seed germination and seedling establishment in alien-cleared sites. The novelty of this study is that it focuses on species targeted for use in large-scale lowland fynbos restoration with respect to alien cleared sites.

The drought/desiccation tolerances of seed germinants and newly emerged seedlings of CFSF species across different moisture regimes were investigated. Herewith, post-germination drought/desiccation survival refers to the ability of the radicle to expand until coleoptile stage prior to emergence after repeated drying and re-wetting, whereas seed germination refers to radicle emergence of at least 2 mm.

4.2. MATERIALS AND METHODS

4.2.1. Germination experiments

4.2.1.1. Laboratory experiments

To investigate seed and seedling drought/desiccation tolerance, drought/desiccation laboratory experiments were performed using conditions mimicking post-fire winter germination periods in fynbos (Mustart and Cowling, 1993a). The experiment ran from March to late October 2014. Seeds of species from different families were selected from Cape Flats Sand Fynbos (Table 1.1).

Seeds were assessed for their ability to germinate judging by radicle emergence of at least 2 mm. Seeds were also assessed for the ability of the radicle to expand and continue growing after repeated drying and re-wetting events. The results found in Chapter 2 and 3 were used to select the best germination conditions and pre-germination treatments for species in this experiment. Wherein, in order to overcome dormancy, all seeds were pre-treated using a freshly prepared batch of smoke water solution prepared by Kirstenbosch Botanical Gardens. One hundred ml of smoke water concentrate was diluted with 900 ml of distilled water to make 1000 ml standard concentration of 10% using a graduated cylinder. Seeds were soaked for 24 hours at room temperature. For hard-coated seeds, a heat-pulse pre-germination treatment was applied: constant 100 °C was maintained in an oven and seeds were treated for 5 minutes. Seeds were left to cool off under room temperature and then soaked in smoke water solution for 24 hours (as in Chapter 2).

The following drying-down treatments were applied according to Mustart et al. (2012) to 160 seeds of each species (4 Petri dish replicates with 40 seeds each):

- i) Experiment 0: Control treatment - kept moist in covered Petri dishes.
- ii) Experiment 1: pre-germination desiccation: Kept moist with 3ml distilled water until just before radicle emergence based on the germination rate of previous year's experiment (Chapter 2 results) followed by a desiccation wherein the cover was removed for 21 days, and then a rewetting with 3ml distilled water in covered Petri dishes for 3 days .

- iii) Experiments 2-5: Post-germination desiccation: kept moist until radicle emergence of 2 mm followed by four different desiccation treatments of 6, 13, 18 and 25 days respectively, and later 3 days rewetting in covered Petri dishes. Desiccation and rewetting was repeated several times mimicking the natural rainfall pattern (during the April to September rainfall season).

Petri dishes were sealed in plastic polythene bags, closed with plastic clips (Keeley and Fotheringham, 1997; Holmes and Newton, 2004), and put in Stellenbosch University germination chambers in stacks of eight Petri dishes with alternating temperatures of 21° C /10° C and a photoperiod of 10h L/14hD in. Seeds were placed on two layers of filter paper with 3 ml distilled water and 0.1% benlate solution (fungicide) in Petri dishes. Each Petri dish was marked by labelling the species name, treatment and replication number. Experimental desiccation followed by wetting mimicked the post-fire rain cycle in the field (Bond, 1984; Brits, 1986; Mustart and Cowling, 1993b) as per recorded rainfall data obtained from the Cape Town Weather Observation station (1994-2014). Seed germination was monitored on the 7th day of the experiment by counting and recording germinated seeds, thereafter seeds were monitored every 3rd day. The experiment ran for a minimum of 161 days, but only three germination values are shown on the results and not the germination progress curve.

4.2.1.2. Greenhouse experiments

To investigate seedling desiccation tolerance, a greenhouse experiment was conducted in a solid roof greenhouse at Stellenbosch University. Greenhouse studies do not duplicate field conditions, yet they do provide information on the broad range of responses of species to water deficiency (Frasier et al., 1984, 1985). However, the greenhouse microhabitat resembled field conditions (i.e., fluctuating diurnal daily temperatures and winter-spring temperatures). Seeds of the Cape Flats Sand Fynbos species in Table 1.1 were used and pre-treated as for those in the laboratory experiment. The experiment ran from June to November 2014.

To determine a moisture regime that resembles the conditions in the field, rainfall data records from Cape Town Weather Observation station (data for station [0021178A3]) were used. Rainfall data were analysed by extracting the number of dry (rain free) periods from April to August. This is because the main germination period is in this winter rainfall period, whereas September is too late for germination and successful seedling establishment before the hot, dry summer. It was assumed that a significant rainfall event over several days with a total rainfall of >50mm could trigger germination. The rain-free days following the first significant rains (years versus number of rain-free day periods in winter- i.e., from one to 30 days of the maximum drought periods in the dataset) were recorded in an excel spreadsheet. The number of times at which each rain-free period was encountered for each year after first significant rain until August was recorded and a frequency distribution produced. Then, the relationship between long drought periods and total winter rainfall was assessed to obtain watering regime data (Figure 4.1). The information was used to guide the establishment of watering regimes for the desiccation nursery experiment.

Four replicate seed trays of 40 seeds (4 replicates \times 40 seeds) were used for the greenhouse experiment (to ensure that I had enough seedlings, given the low seed germination in previous chapters, I planted an extra 10 seeds in each tray although I only considered 40). Large seeds were buried in the soil in rows at 2 cm depth, but small seeds were mixed with sand and sprinkled evenly on the soil in seed trays. These were watered with a calibrated irrigation system. Seed trays, 300 \times 270 \times 100 mm, were lined with a single layer of non-woven fabric cleaning cloth, filled to a depth of 100 mm with a layer of river washed sand (30 mm) and covered in a layer of field soil (70 mm) collected from molerat mounds adjacent to Blaauwberg Nature Reserve. All trays were thoroughly soaked and kept moist for the average time required to initiate germination. After emergence, watering was withheld for three treatments:

- A moist regime (i.e., control) where the seeds did not dry out with watering at least every 3 days,
- Intermediate desiccation, wherein seeds were watered at an interval of 12 days.
- Severe desiccation, wherein seeds were watered after every 30 days.

All trays were weeded of non-target species. Seedlings of target species were counted fortnightly and marked with toothpicks. Seedling trays were moved around at random every second week to reduce the influence of any erratic delivery of irrigation water and ensuring that all trays were exposed to the same conditions in the greenhouse. The experiment ran for a minimum of 161 days, but only three germination values are shown on the results and not the germination progress curve.

4.2.2. Statistical analysis

For each species, differences in numbers of germinants and numbers of seedlings (for growth chamber and greenhouse experiments respectively) between the different treatments (i.e. desiccation and re-watering) were analysed using repeated-measures Analysis of Variance. Bonferroni post-hoc multiple comparisons were used to compare significance between treatments. In the tables, different letters have been used to indicate significant differences between species and their treatments. Least squared means graphs were generated to show the trends in the response of the different species to desiccation. STATISTICA 12 software was used to calculate all summary statistics and for the above statistical tests. All tests were conducted at a significance level of 5%.

Ordination analysis (Principle component Analysis (PCA)) was used to determine variance in species response to different desiccation treatments (i.e. control, 6, 13, 18, 21 and 25 days) for each of the initial germination (pre), desiccation (dry) and rewatering (rew) respectively. The amount of variance (the spread of data values) in the samples was determined using biplots to see a response pattern between treatments and explanatory variables (regeneration mode, growth form and seed size). PRIMER 6 software was used to run this analysis.

4.3. RESULTS

4.3.1. Growth chamber experiment

4.3.1.1. Pre-germination desiccation

The initial experiment was performed with 17 species (Table 1.1) but only 15 species germinated with overall results of the repeats and treatments ($F(2,236) = 27.665$, $P < 0.001$) (Figure 4.2. a). Hence, *Phyllica cephalantha* and *Wachendorfia multiflora* were removed from the dataset. Desiccation of seeds before germination (pre-germination desiccation) had no significant negative effect on germination of thirteen species (i.e. *Erica plumosa*, *Serruria fasciflora*, *Passerina corymbosa*, *Metalasia densa*, *Chrysocoma ciliata*, *Senecio elegans*, *Watsonia meriana*, *Leucadendron salignum*, *Erica mammosa*, *Agathosma imbricata*, *Protea scolymocephala*, *Protea repens* and *Thamnochortus punctatus*), whereas two species survived pre-germination desiccation with lower germinants after rewatering (i.e. *Ursinia anthemoides* and *Diosma oppositifolia*) (Table 4.1, Figure 4.2).

In pre-germination desiccation species showed various responses to rewatering, both positive and negative. Nine species responded positively to rewatering with significant increases in numbers of germinants: *Senecio elegans*, *Erica mammosa*, *Serruria fasciflora*, *Passerina corymbosa*, *Watsonia meriana*, *Protea repens*, *Metalasia densa*, *Erica mammosa*, and *Agathosma imbricata* (Table 4.1). *Thamnochortus punctatus* showed significant differences in numbers of survived germinants between initial germination, desiccation and rewatering, with a decrease in germinants after desiccation followed by an increase in seedlings revival after rewatering (Table 4.1). *Leucadendron salignum* and *Protea scolymocephala* showed significant decreases in the number of germinants after desiccation, whilst rewatering did not lead to a significant increase in germinants (Table 4.1). *Chrysocoma ciliata* had a significantly higher number of germinants before desiccation compared with rewatering and desiccation (Table 4.1). *Ursinia anthemoides* and *Diosma oppositifolia* had a negative response to rewatering with significantly lower numbers of germinants after desiccation (Table 4.1).

4.3.1.2. Post-germination desiccation

Overall, 15 of 17 species germinated (Table 4.2) and significant differences between treatments and species with overall results of the repeats and treatments ($F(8,590) = 27.105$, $P < 0.0001$) were recorded. Short periods of desiccation generally had a negative effect on germinant survival as observed when comparing the 6 days treatment with the longest desiccation of 25 days (Figure 4.2. b). Most species recovered from at least one desiccation treatment. For all treatments and all species, desiccation resulted in significant decreases in numbers of emerged coleoptile, but with most of the species recovering to pre-desiccation germination levels after rewatering (Table 4.2, Figure 4.2. b and 4.4. a, e).

Leucadendron salignum had a significant number of germinants before and after desiccation at 25 days (Table 4.2). *Ursinia anthemoides* and *Senecio elegans* had an increase in germinant numbers after desiccation and rewatering at 6, 18 and 25 days (Table 4.2). *Thamnochortus punctatus* and *Metalasia densa* had a decreased number of germinants after desiccation, but then an increase of germinants after rewatering at 18 and 25 days (Table 4.2). *Erica mammosa*, *Serruria fasciflora*, *Passerina corymbosa*, *Watsonia meriana*, *Protea scolymocephala*, *Protea repens*, *Metalasia densa*, *Erica plumosa* and *Agathosma imbricata* also had a decreased number of germinants after desiccation, but then had a significant increase in germinants after rewatering at 6, 13, 18 and 25 days (Table 4.2). *Chrysocoma ciliata* and *Leucadendron salignum* had an increase in number of germinants with rewatering after a decrease in desiccation at 6, 13 and 25 days (Table 4.2, Figure 4.4. a, e).

4.3.2. Greenhouse experiment

Overall, seeds of all species germinated well with overall results of the repeats (i.e. pre, dry, rew) and treatments (i.e. 12 days, 30 days) (Table 4.3 and Figure 4.3 and 4.6) and significant differences among treatments and species ($F(4,402)=216.87$, $P<0.0001$) were recorded. For the 12 and 30 days, treatments and all species' seedlings survived desiccation after rewatering (Table 4.3). *Chrysocoma ciliata*, *Diosma oppositifolia*, *Senecio elegans*, *Serruria fasciflora* and *Thamnochortus punctatus* had a significantly higher number of seedlings after rewatering compared to both initial germination and desiccation at 30 days (Table 4.3) whilst the other species had no significant increase after desiccation.

To test whether there was a correlation between germination response and explanatory variables (i.e. regeneration mode, growth form and seed size) an ordination analysis (PCA) was conducted. Overall all the graphs showed a variation of not more than 60% at PCA 1 compared to PCA 2 which was not more than 30% of the total variation. PCA showed that species were grouped according to treatments (i.e. initial germination, desiccation, rewatering), indicating that species' germination patterns were strongly correlated with treatments. However, these grouping could not be correlated to any of the explanatory variables for most of the species. Only five species (*Metalasia densa*, *Serruria fasciflora*, *Thamnochortus punctatus*, *Passerina corymbosa* and *Agathosma imbricata*) to regeneration mode, growth forms and seed size (Figure 4.4.).

4.4. DISCUSSION

4.4.1 Species response to desiccation

This study shows that desiccation in both pre and post-germination of Cape Flats Sand Fynbos (CFSF) species is not a major cause of lack of seedling emergence and establishment. This finding corresponds with the findings of Mustart et al. (2012) who found that desiccation tolerant species such as *Protea repens* are less likely to be impacted by climate aridification. Similarly, Stock et al. (1992) pointed out that water is not a limiting factor in fynbos. “No one size fits all” unless otherwise research is conducted on both nutrient-poor and nutrient-rich fynbos, since Miller (1985) showed that water appears to act as a selective pressure in the fynbos region only on nutrient-rich soils. In *Acacia*, invaded soils there are nutrient rich soils that are higher than normal fynbos levels (Campbell, 1985) that might account for low germination of indigenous plant species.

In post-germination treatments there was generally an effect on mortality after the shorter desiccation periods (6 days), wherein species seemed to have tolerated desiccation in longer periods than in the shorter period. This is explained by the sclerophous plants of the fynbos that are resistant to the harsh Mediterranean-type conditions. Sclerophylly is a summer-drought strategy that is prominent in systems with low nutrients. This lack of nutrients leads to carbon-rich plants with desiccation tolerance. Fynbos sclerophylly includes proteoids, ericoids, restioids (Le Maître and Midgley, 1992). Both greenhouse and growth chamber experiments showed less overall effect of desiccation tolerance, but did not identify significant differences among desiccation treatments, which consent with Jacobsen et al. (2007) who found that there was convergence of community and species-specific water stress “strategies” with respect to their xylem traits. This is in contrast to the Mustart et al. (2012) study who found that the longer the desiccation period, the greater the impact on seedling mortality. Although, she also found that in some cases as little as six days desiccation periods resulted in a major decline in seed survival, in this study’s case the results differed with the current study since both moist and dry habitats. The relatively high number of germinants and seedlings that survived after desiccation and rewatering, compared to the control, implies that desiccation has a limited effect on CFSF species.

Although most of the species were desiccation tolerant, a few were unevenly sensitive to the desiccation treatments. It appears that desiccation mortality does not play a major role in Sand Fynbos seedling establishment. Germinants were tolerant to extreme desiccation up to the stage of emergence of the first leaf from the coleoptile. Thus, mortality of germinants and seedlings in the field may rather be due to other constraints such as granivory, herbivory, soil chemical properties, soil microbial community and elevated nutrient levels.

Bond (1984) showed that South Swartberg Sandstone Fynbos vegetation seeds planted in rodent-proof, shade-cloth enclosures, germinated earlier and in greater numbers than those planted in the open. Ten weeks after planting, 95% of the viable seed had germinated in the enclosures, compared with only 12% in the controls.

Another constraint was changes in soil chemical properties. It has been shown that invasion by nitrogen fixing *Acacia* species does lead to alterations in soil nutrient cycling which negatively affects indigenous species establishment (Mehta, 2000; Yelenik et al., 2004). Higher levels of soil nitrogen is said to accelerate the growth of other invasive species such as weedy grasses (i.e. *Ehrharta calycina*) (Jovanovic et al., 2009) which tend to outcompete less competitive fynbos species.

Seedling establishment in alien-cleared CFSF sites might also be influenced by microbial activities associated with the alien species. Slabbert et al. (2014) showed that the bacterial changes in soil microbial community composition could be linked to *Acacia* invasion. The presence of invasive *Acacia* was correlated with specific bacterial phyla that do not favour the establishment of indigenous fynbos species. However, high similarity between cleared and original sites suggests that this effect on the soil bacterial community structure may not be permanent.

Other factors could be linked to inappropriate restoration approaches after clearing e.g. lack of pre-germination seed treatments. The significantly high germination of the target CFSF species in greenhouse experiments conducted with pre-germination treatments compared to field experiments (lacking pre-germination treatments) provides evidence that pre-germination treatments ought to be included in order to yield reliable results.

4.4.2. Implication for restoration

The vital study finding is that species of the Cape Flats Sand Fynbos are desiccation tolerant despite their growth form. This supports the motion that fynbos' nutrient-poor status is compounded by summer drought and winter temperatures and both fynbos and Kwongan plants possess specialised desiccation tolerance (Branch, 1988; Cowling, 1992; Richards et al. 1997).

Drought/desiccation is unlikely to be the cause of low seed germination and seedling emergence in the field. This implies that there are other factors that might be linked to low fynbos recovery (i.e. granivory, herbivory, elevated nutrient-levels, soil microbial communities or a combination thereof).

4.4.3. Research need

For future research, it is therefore recommended that granivory and herbivory needs to be minimised to ensure success in the field. Thomas and Davis (1990) concluded that seedling ability to survive drought is of much more importance than initial seedling numbers; therefore, there is a need for further research on post-fire mortality and water relations of the CFSF species in the field. Indigenous CFSF species may be deprived of the resources required for germination and seedling establishment by the more competitive *Acacia* (Rowntree 1991). *Acacia saligna* transforms the quality of the soil to better suit its own needs (Richardson et al., 1997). Its large biomass allows it to create large amounts of litterfall, which results in addition of nutrients and input of organic matter that indigenous fynbos species are unable to uptake such nutrients effectively since they lack mycorrhizal relationships (Cowling, 1992). This is in comparison with a study by Musil (1993) who found that plants from infertile sites like *Protea repens* usually have low growth rates, low nutrient absorption rates and they do not respond to nutrient availability. Hence, a greenhouse alongside field experiment should be done with comparison of water relations competition and nutrients tolerance amongst *Acacia saligna* and indigenous CFSF species.

REFERENCES

- Agenbag, L., 2006. A study on an altitudinal gradient investigating the potential effects of climate change on fynbos and the fynbos-succulent Karoo boundary. MSc thesis, University of Stellenbosch, Stellenbosch.
- Alpert, P., 2005. The limits and frontiers of desiccation-tolerant life. *Integrative and Comparative Biology* 45, 685-695.
- Bell, D.T., 1999. Turner Review No.1: The process of germination in Australian species. *Australian Journal of Botany* 47, 475-517.
- Bond, W.J., 1984. Fire survival of Cape Proteaceae - influence of fire season and seed predators. *Vegetatio* 56, 65-74.
- Branch, G.M., 1988. *Explore the Cape flora and its animals*. Struik publishers, Cape Town.
- Brits, G.J., 1986. The effect of hydrogen peroxide treatment on germination in Proteaceae species with serotinous and nut-like achenes. *South African Journal of Botany* 52, 291-293.
- Brown, N.A.C., 1993. Promotion of fynbos seeds by plant-derived smoke. *New Phytologist*, 123, 575-583.
- Campbell, B. M. 1985. A classification of the mountain vegetation of the fynbos biome. *Memoirs of the Botanical Survey of South Africa*, No. 50. Botanical Research Institute, Department of Agriculture and Water Supply, Pretoria.
- Cowell, C., 2013. Investigating the most favourable seed establishment methods of restoring sand fynbos on old fields. Unpublished Master of Technology thesis, Cape Peninsula University of Technology.
- Cowling, R., Richardson, D., 1992. *Fynbos - South Africa's Unique Floral Kingdom*. Fernwood, Cape Town
- Cowling, R.M. (Ed.), 1992. *The Ecology of Fynbos*. Oxford University Press, UK.
- Cowling, R.M., Richardson, D.M., Mustart, P.J., 1997. Fynbos. In: Cowling, R.M., Richardson, D.M. and Pierce, S.M. (Eds.). *Vegetation of Southern Africa*. Cambridge University Press, New York. pp. 99-123.

- Esler, K.J., Phillips, N., 1994. Experimental effects of water stress on semi-arid Karoo seedlings: implications for field seedling survivorship. *Journal of Arid Environments* 26, 325-337.
- Esler, K.J., Rundel, P.W., Vorster, P., 1999. Biogeography of prostrate-leaved geophytes in semi-arid South Africa: hypotheses on functionality. *Plant Ecology* 142, 105-120.
- Esler, K.J., von Staden, L., Midgley, G.F., 2015. Determinants of the Fynbos/Succulent Karoo biome boundary: insights from a reciprocal transplant experiment. *South African Journal of Botany*. In Press.
- Evans, C.E., Etherington, J.R., 1991. The effect of soil water potential on seedling growth of some British plants. *New Phytologist* 118, 571-579.
- Fisher, J.L., Loneragan, W.A., Dixon, K., Veneklaas, E.J., 2009. Soil seed bank compositional change constrains biodiversity in an invaded species-rich woodland. *Biological Conservation* 142 (2), 256-269.
- Frasier, G.W., Cox, J.R., Woolhiser, D.A., 1984. Emergence and seedling survival of two warm-season grasses as influenced by the timing of precipitation: a greenhouse study. *Journal of Range Management* 37, 7-11.
- Frasier, G.W., Cox, J.R., Woolhiser, D.A., 1985. Emergence and survival responses of seven grasses for six wet-dry sequences. *Journal of Range Management* 38, 372-377.
- Gaertner, M., Richardson, D.M., Privett, S.D.J., 2011. Effects of alien plants on the ecosystem structure and functioning and implications for restoration: insights from three degraded sites in South African fynbos. *Environmental Management* 48(1):57-69.
- Groom, P.K., 2002. Seedling water stress response of two sandplain *Banksia* species differing in ability to tolerate drought. *Journal of Mediterranean Ecology* 3(4), 3-9.
- Harper, J. L., Williams, J. T., Sagar, G. R., 1965. The behaviour of seeds in soil I. The heterogeneity of soil surface and its role in determining the establishment of plants from seeds. *Journal of Ecology* 53, 273-286.
- Holmes, P., Newton, R.J., 2004. Patterns of seed persistence in South African Fynbos. *Plant Ecology* 172,143-158.
- Holmes, P., 2001. Shrubland restoration following woody alien invasion and mining: effects of topsoil depth, seed source, and fertilizer addition. *Restoration Ecology* 9(1), 71-84.

- Holmes, P., 2002. Depth distribution and composition of seed banks in alien-invaded and uninvaded Fynbos vegetation. *Australian Ecology* 27, 110-120.
- Holmes, P., Cowling, R.M., 1997. The effects of invasion by *Acacia Saligna* on the guild structure and regeneration capabilities of South Africa shrublands. *Journal of Applied Ecology* 34, 317-332.
- Holmes, P., Newton, R.J., 2004. Patterns of seed persistence in South African Fynbos. *Plant Ecology* 172, 143-158.
- Holmes, P.M., Foden, W., 2001. The effectiveness of post-fire soil disturbance in restoring fynbos after alien clearance. *South African Journal of Botany* 67, 533-539.
- Holmes, P.M.; Richardson, D.M.; van Wilgen, B.W., Gelderblom, C., 2000. Recovery of South African fynbos vegetation following alien woody plant clearing and fire: implications for restoration. *Australian Ecology* 25, 631-639.
- Jacobsen, A.L., Agenbag, L., Esler, K.J., Pratt, R.B., Ewers, F.W., Davis, S.D., 2007. Xylem density, biomechanics and anatomical traits correlate with water stress in 17 evergreen shrub species of the Mediterranean-type climate region of South Africa. *Journal of Ecology* 95:171-183.
- Jacobsen, A.L., Esler, K.J., Pratt, R.B., Ewers, F.W., 2009. Water stress tolerance of shrubs in Mediterranean-type climate regions: converge of fynbos and succulent Karoo communities with California shrub communities. *American Journal of Botany* 96(8), 1445-1453.
- Jones, R. H., Allen, B. P., Sharitz, R. R., 1997. Why do early emerging tree seedlings have survival advantages?: A test using *Acer rubrum* (Aceraceae). *American Journal of Botany* 84, 1714 – 1718.
- Jovanovic, N.Z., Israel, S., Tredoux, G., Soltau, L., Le Maître, D., Rusing, F., Rozanov, A., van der Merwe, N., 2009. Nitrogen dynamics in land cleared of alien vegetation (*Acacia saligna*) and impacts on groundwater at Riverlands Nature Reserve (Western Cape) *Water SA* (Pretoria). 35(1), 37-44.
- Keeley, J.E., Bond, W.J., 1997. Convergent seed germination in South Africa fynbos and Californian chaparral. *Plant ecology* 133, 153-167.

- Keeley, J.E., Fotheringham, C.J., 1997. Trace gas emissions and smoke-induced seed germination. *Science* 276, 1248-1250.
- Kraaij, T., Cramer, M.D. 1999. Do the gas exchange characteristics of alien acacias enable them to successfully invade the fynbos? *South African Journal of Botany* 65(3), 232-238.
- Kruger, F. J., Mitchell, D. T., Jarvis, J. U. M. (Eds.), 1983. *Mediterranean-Type Ecosystems. The role of nutrients.* Springer-Verlag, Berlin.
- Lamont, B. B., 1983. Strategies of maximizing nutrient uptake in two Mediterranean ecosystems of low nutrient status. pp 246–273. In: Kruger, F. J., Mitchell, D. T., Jarvis, J. U. M. (Eds.) *Mediterranean-Type Ecosystems – the role of nutrients.* Springer-Verlag, Berlin.
- Lamont, B.B., Enright, N.J., Bergl, S.M., 1989. Coexistence and competitive exclusion of *Banksia hookeriana* in the presence of congeneric seedlings along a topographic gradient. *Oikos* 56, 39-42.
- Le Maître, D.C., 2004. Predicting invasive species impacts on hydrological processes: the consequences of plant physiology for landscape processes. *Weed Technology* 18, 1408-1410.
- Le Maître, D.C., Midgley, J.J., 1992. Plant reproductive. In: Cowling, R.M. (Ed.). *The Ecology of Fynbos: Nutrient, Fire and Diversity.* Cape Town, Oxford University press. pp. 135-174.
- Mack, R.N., 1976. Survivorship of *Cerastium atrovirens* at Aberffraw, Anglesey. *Journal of Ecology* 64, 309-312.
- Maze, K. E., Bond, W. J., 1996. Are *Protea* populations seed limited? Implications for wildflower harvesting In Cape fynbos. *Austral Ecology* 21, 96–105.
- Mehta, S., 2000. The invasion of South African fynbos by an Australian immigrant: The story of *Acacia saligna*. *Restoration and Reclamation Review* 6(5), 1-10.

- Midgley, G.F., Chapman, R.A., Hewitson, B., Johnston, P., de Wit, M., Ziervogel, G., Mukheibir, P., van Niekerk, L., Tadross, M., van Wilgen, B.W., Kgope, B., Morant, P.D., Theron, A., Scholes, R.J., Forsyth, G.G., 2005. *A Status Quo, Vulnerability and Adaptation Assessment of the Physical and Socio-economic Effects of Climate Change in the Western Cape*. Report to the Western Cape Government, Cape Town, South Africa. CSIR Report No. ENV-S-C 2005-073, Stellenbosch.
- Midgley, G.F., Hannah, L., Millar, D., Rutherford, M.C., Powrie, L.W., 2002. Assessing the vulnerability of species richness to anthropogenic climate change in a biodiversity hotspot. *Global Ecology and Biogeography* 11, 445-451.
- Midgley, J.J., 1988. Mortality of Proteaceae seedlings in their first summer. *South African Journal of Forestry* 145(1), 9–12.
- Midgley, S.J., Turnbull, J.W., 2003. Domestication and use of Australian acacias: Case studies of five important species. *Australian Systematic Botany* 16(1), 89
- Miller, J.M., 1985. Plant water relations along a rainfall gradient between the Succulent Karoo and Mesic Mountain Fynbos in the Cedarberg Mountains near Clanwilliam, South Africa. M.Sc thesis, University of Cape Town.
- Milton, S.J., Hall, A.V., 1981. Reproductive biology of Australian Acacias in the South – Western Cape Province, South Africa. *Transactions of the Royal Society of South Africa* 44, 465-485.
- Mitchell, D. T., Coley, P. G. F., Webb S., Allsopp, N., 1986. Litterfall and decomposition processes in the coastal fynbos vegetation, south-western Cape, South Africa. *Journal of Ecology* 74, 977–993.
- Morris, T.L., Esler, K.J., Barger, N.N., Jacobs, S.M., Cramer, M.D., 2011. Ecophysiological traits associated with the competitive ability of invasive Australian acacias. *Diversity and Distributions*, 17, 898-910.
- Musil, C.F., 1993. Effect of invasive Australian acacias on the regeneration, growth and nutrient chemistry of South African lowland fynbos, *Journal of Applied Ecology* 30(2), 361-372.
- Mustart, P.J., Cowling, R.M., 1991. Seed germination of four serotinous Agulhas plain Proteaceae. *South African Journal of Botany* 57, 310-13.

- Mustart, P.J., Cowling, R.M., 1993a. Effects of soil and seed characteristics on seed germination and their possible roles in determining field emergence patterns of four Agulhas Plain (South Africa) Proteaceae. *Canadian Journal of Botany* 71, 1363-1368.
- Mustart, P.J., Cowling, R.M., 1993b. The role of regeneration stages in the distribution of edaphically restricted fynbos Proteaceae. *Ecology* 74, 1490–1499.
- Mustart, P.J., Cowling, R.M., Wright, M.G., 1995. Clustering of fertile seed in infructescences of serotinous *Protea* species: an anti-predation mechanism? *African Journal of Ecology* 33, 224-9.
- Mustart, P.J., Rebelo, A.G., Juritz, J., Cowling, R.M., 2012. Wide variation in post-emergence desiccation tolerance of seedlings of Proteoid shrubs. *South African Journal of Botany* 80, 110-117.
- Pasquet-Kok, J., Creese, C., Sack, L., 2010. Turning over a new ‘leaf’: multiple functional significances of leaves versus phyllodes in Hawaiian *Acacia koa*. *Cell and Environment* 33, 2084-2100.
- Peperkorn, R., Werner, C., Beyschlag, W., 2005. Phenotypic plasticity of an invasive acacia versus two native Mediterranean species. *Functional Plant Biology* 32, 933-944.
- Reader, R.J., 1991. Relationship between seedling emergence and species frequency on a gradient of ground cover density in an abandoned pasture. *Canadian Journal of Botany* 69, 1397-1401.
- Rebelo, A.G., Boucher, C., Helme, N.A., Mucina, L., Rutherford, M.C., 2006. Fynbos biome. In: Mucina, L., Rutherford M.C., (Eds.). *The Vegetation of South Africa, Lesotho and Swaziland: Strelitzia*, 19, pp. 52–219.
- Rebelo, A.G., Holmes, P.M., Dorse, C., Wood, J., 2011. Impacts of urbanization in a biodiversity hotspot: Conservation challenges in Metropolitan Cape Town. *South African Journal of Botany* 77(1), 20-35.
- Richards, M.B., Stock, W.D., Cowling, R.M., 1997. Soil nutrient dynamics and community boundaries in the fynbos vegetation of South Africa. *Plant Ecology* 130, 143-153.
- Richardson, D.M., MacDonald, I.A.W., Hoffmann, J.H., Henderson L., 1997. Alien plant invasions. In Cowling, R.M., Richardson, D.M., Pierce, S.M. (Eds.). *Vegetation of Southern Africa*. Cambridge University Press, Cambridge, UK. pp. 535-570.

- Richardson, D.M., van Wilgen, B.W., 2004. Invasive alien plants in South Africa: how well do we understand the ecological impacts? *South African Journal of Science* 100, 45-52.
- Roche, B.F., Roche, C.T., Chapman, R.C., 1994. Impacts of grassland habitat on yellow starthistle (*Centaurea solstitialis* L.) invasion. *Northwest Science* 68, 86-96.
- Rowntree, K.M., 1991. An assessment of the potential impact of alien invasive vegetation on the geomorphology of river channels in South Africa. *South African Journal of Aquatic Science* 17, 28-43.
- Slabbert, E., Jacobs, S.M., Jacobs, K., 2014. The Soil Bacterial Communities of South African Fynbos Riparian Ecosystems Invaded by Australian Acacia Species. *PLoS ONE* 9(1): e86560. doi:10.1371/journal.pone.0086560
- Smith, R.E., van Wilgen, B.W., Forsyth, G.G., Richardson, D.M. 1992. Coexistence of seeders and resprouters in a fire-prone environment: the role of ecophysiology and soil moisture. In: van Wilgen, B.W., Richardson, D.M., Kruger, F.J., van Hensbergen, H.J., (Eds.). *Fire in South African mountain fynbos: Ecosystem, community and species response of Swartboskloof*. Springer-Verlag, Berlin Heidelberg.
- StatSoft Inc., 2015. STATISTICA (Data Analysis Software System), version 12. www.statsoft.com. Tulsa, Oklahoma.
- Stock, W.D., van der Heyden, F., Lewis, O.A.M., 1992. Plant structure and function. In: Cowling, R.M., (Ed.). *The Ecology of Fynbos: Nutrients, Fire and Diversity*. Oxford University Press, Cape Town.
- Thomas, C.M., Davis, S.D., 1989. Recovery patterns of three chaparral shrub species after wildlife. *Oecologia* 80, 309-320.
- Thuiller, W., Lavorel, S., Midgley, G., Lavergne, S., Rebelo, T., 2004. Relating plant traits and species distributions along bioclimatic gradients for 88 *Leucadendron* taxa. *Ecology* 85(6), 1688-1699.
- Turner, N.C., 1986. Adaptation to water deficits: a changing perspective. *Australian Journal of Plant Physiology* 13, 175-190.

- van Hensbergen, H.J., Botha, S.A., Forsyth, G.G., Le Maitre, D.C. 1992. Do small mammals govern vegetation recovery after fire in fynbos? In: van Wilgen, B.W., Richardson, D.M., Kruger, F.J., van Hensbergen, H.J., (Eds.). Fire in South African Mountain fynbos: Ecosystem, community and species response at Swartboskloof. Berlin: Springer-Verlag, pp. 182–202.
- van Wilgen, B.W., Forsyth, G.G., 1992. Regeneration strategies in fynbos plants and their influence on the stability of community boundaries after fire. In: van Wilgen, B.W., Richardson, D.M., Kruger, F.J. and van Hensbergen, H.J., (Eds.). Fire in South African mountain fynbos: Ecosystem, community and species response of Swartboskloof. Springer-Verlag, Berlin Heidelberg.
- van Wilgen, B.W., van Hensbergen, H.J., 1992. Fuel properties of vegetation in Swartboskloof in Ecological Studies. In: van Wilgen, B.W., Richardson, D.M., Kruger, F.J., van Hensbergen, H.J., (Eds.). Fire in South African Mountain Fynbos. Springer-Verlag, Berlin, pp. 38-53.
- West, A.G., Dawson, T.E., February, E.C., Midgley, G.F., Bond, W.J., Aston, T.L., 2012. Diverse functional response to drought in a Mediterranean-type shrubland in South Africa. *New Phytologist* 195, 396-407.
- Whelan, R.J., 1995. *The Ecology of fire*. Cambridge University Press, Cambridge.
- Witkowski, E.T.F., 1991. Growth and Competition between Seedlings of *Protea-Repens* (L) L and the Alien Invasive, *Acacia Saligna* (Labill) Wendl in Relation to Nutrient Availability. *Functional Ecology* 5, 101-110.
- Yelenik, S.G, Stock, W.D., Richardson, D.M., 2004. Ecosystem level impacts of invasive *Acacia saligna* in the South African Fynbos. *Restoration Ecology* 12(1), 45-51.

Table 4.1: Effects of different pre-germination desiccation treatments on fifteen indigenous CFSF species tested under growth chamber conditions (with alternating temperatures of 21° C/ 10° C and a photoperiod of 10h L/ 14h D). Data are untransformed means \pm SE of survived germinants(%), $n=4 \times 40$ seeds) and results of repeated measures ANOVA values are shown (* $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$). The repeats of initial germination (pre), desiccation (dry) and rewatering (rew) maximum response in different treatments (i.e. control and 21 days). Superscript letters show where the significant differences lie between the species and treatments after a Bonferroni post-hoc test, and those not significantly different are indicated by the different letters.

	Control	Pre-germination desiccation 21 days			
Species name	Control	pre	dry	rew	ANOVA=F(2,12)
<i>Agathosma imbricata</i>	80.63 \pm 5.81 ^a	58.75 \pm 5.81 ^a	14.38 \pm 4.07 ^b	30.00 \pm 6.31 ^b	26.469 ^{***}
<i>Chrysocoma ciliata</i>	62.50 \pm 5.92 ^a	0.63 \pm 5.92 ^b	82.50 \pm 8.35 ^a	72.50 \pm 7.71 ^a	84.646 ^{***}
<i>Diosma oppositifolia</i>	29.38 \pm 6.10 ^{ab}	27.50 \pm 6.10 ^a	11.25 \pm 6.33 ^b	11.88 \pm 6.16 ^b	8.837 ^{**}
<i>Erica mammosa</i>	90.63 \pm 4.64 ^a	80.00 \pm 4.64 ^a	18.13 \pm 3.44 ^c	29.38 \pm 4.43 ^b	144.68 ^{***}
<i>Erica plumosa</i>	67.50 \pm 3.61 ^a	52.50 \pm 3.61 ^a	6.25 \pm 2.55 ^c	19.38 \pm 3.01 ^b	54.311 ^{***}
<i>Leucadendron salignum</i>	26.88 \pm 5.24 ^{ab}	18.13 \pm 5.24 ^a	8.13 \pm 5.24 ^b	115.63 \pm 6.16 ^{ab}	5.778 [*]
<i>Metalasia densa</i>	46.88 \pm 12.58 ^{ab}	63.75 \pm 12.58 ^a	19.38 \pm 6.03 ^b	26.25 \pm 6.61 ^b	11.148 ^{***}
<i>Passerina corymbosa</i>	83.13 \pm 4.91 ^a	67.50 \pm 4.91 ^a	8.75 \pm 3.53 ^b	15.63 \pm 3.66 ^b	72.246 ^{***}
<i>Protea repens</i>	61.88 \pm 5.98 ^{ab}	56.88 \pm 5.98 ^a	16.25 \pm 5.44 ^c	35.00 \pm 5.79 ^b	19.479 ^{***}
<i>Protea scolymocephala</i>	67.50 \pm 5.40 ^a	45.00 \pm 5.40 ^a	12.50 \pm 5.10 ^b	22.50 \pm 5.68 ^b	13.759 ^{***}
<i>Senecio elegans</i>	99.38 \pm 6.94 ^a	80.00 \pm 6.94 ^a	11.88 \pm 2.25 ^b	27.50 \pm 0.85 ^b	40.362 ^{***}
<i>Serruria fasciflora</i>	60.00 \pm 7.48 ^a	41.88 \pm 7.48 ^a	7.50 \pm 4.89 ^b	16.25 \pm 4.87 ^b	15.161 ^{***}
<i>Thamnochortus punctatus</i>	82.50 \pm 4.39 ^a	55.00 \pm 4.39 ^b	16.88 \pm 4.04 ^d	30.00 \pm 3.75 ^c	33.894 ^{***}
<i>Ursinia anthemoides</i>	99.38 \pm 0.63 ^a	99.38 \pm 0.63 ^a	95.63 \pm 0.95 ^a	38.75 \pm 5.72 ^b	65.010 ^{***}
<i>Watsonia meriana</i>	99.38 \pm 4.07 ^a	88.13 \pm 4.07 ^{ab}	95.00 \pm 0.72 ^a	81.88 \pm 2.92 ^b	4.138 [*]

Table 4.2: Effects of different post-germination desiccation treatments on fifteen indigenous CFSF species tested under growth chamber conditions (with alternating temperatures of 21° C/ 10° C and a photoperiod of 10h L/ 14h D). Data are untransformed means ± SE of survived germinants $n=4 \times 40$ seeds in percentages and results of repeated measures ANOVA values are shown (* $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$, NS is not significant $P > 0.05$). The repeats of initial germination (pre), desiccation (dry) and rewatering maximum response in different treatments (i.e. 6, 13, 18, and 25 days).

Species name	Post germination desiccation												ANOVA = F(8,30)
	6 days			13 days			18 days			25 days			
	pre	dry	rew	pre	dry	rew	pre	dry	rew	pre	dry	rew	
<i>A. imbricata</i>	48±5.66 ^b	14±2.91 ^d	25±4.35 ^{dc}	44±5.66 ^{bc}	21±2.91 ^d	23±4.35 ^d	53±5.66 ^b	23±2.91 ^{dc}	23±4.35 ^{dc}	66±5.66 ^{ab}	14±2.91 ^d	21±4.35 ^{dc}	8.352 ^{***}
<i>C. ciliata</i>	89±10.43 ^a	7±5.87 ^f	54±9.61 ^{abcde}	80±10.43 ^a	8±5.87 ^{fe}	58±9.61 ^{abcd}	71±10.43 ^{ab}	31±5.87 ^{hb}	29±9.61 ^{fc}	54±10.43 ^{af}	14±5.87 ^{fd}	26±9.61 ^{hb}	5.931 ^{***}
<i>D. oppositifolia</i>	38±6.18 ^a	16±6.40 ^a	26±5.27 ^a	38±6.18 ^a	21±6.40 ^a	14±5.27 ^a	29±6.18 ^a	10±6.40 ^a	13±5.27 ^a	34±6.18 ^a	11±6.40 ^a	16±5.27 ^a	1.586 ^{ns}
<i>E. mammosa</i>	85±3.43 ^a	18±2.48 ^{dc}	28±6.19 ^{bd}	89±3.43 ^a	18±2.5 ^{48c}	44±6.19 ^b	99±3.43 ^a	19±2.48 ^d	38±6.19 ^{bc}	84±3.43 ^a	20±2.48 ^{dc}	34±6.19 ^{bd}	23.935 ^{***}
<i>E. plumosa</i>	69±5.44 ^a	15±2.68 ^d	38±3.41 ^{cb}	58±5.44 ^{ab}	6±2.68 ^d	15±3.41 ^d	48±5.44 ^{ab}	10±2.68 ^d	21±3.41 ^{cd}	51±5.44 ^{ab}	10±2.68 ^d	14±3.41 ^d	10.817 ^{***}
<i>L. salignum</i>	86±2.76 ^a	12±2.02 ^d	48±6.00 ^b	91±2.76 ^a	96±2.02 ^a	53±6.00 ^b	96±2.76 ^a	96±2.02 ^a	34±6.00 ^{bc}	98±2.76 ^a	92±2.02 ^a	25±6.00 ^{dc}	36.695 ^{**}
<i>M. densa</i>	68±9.60 ^{ab}	19±4.20 ^{ecd}	29±5.12 ^{ecd}	80±9.60 ^a	18±4.20 ^{eb}	33±5.12 ^{eb}	51±9.60 ^{abd}	14±4.20 ^e	24±5.12 ^{ec}	57±9.60 ^{ab}	15±4.20 ^{ed}	23±5.12 ^{ed}	7.483 ^{***}
<i>P. corymbosa</i>	76±7.59 ^a	23±2.87 ^{bc}	34±3.34 ^b	72±7.59 ^a	84±2.87 ^{bc}	14±3.34 ^{bc}	79±7.59 ^a	21±2.87 ^{bc}	28±3.34 ^{bc}	62±7.59 ^a	6±2.87 ^c	18±3.34 ^{bc}	8.218 ^{***}
<i>P. repens</i>	47±6.05 ^{ad}	9±4.00 ^{hf}	26±4.26 ^{ebcg}	53±6.05 ^{ab}	13±4.00 ^{eh}	23±4.26 ^{ehcd}	48±6.05 ^{ab}	10±4.00 ^{hg}	30±4.26 ^{ebdf}	63±6.05 ^a	14±4.00 ^{eh}	26±4.26 ^{ehbcd}	14.028 ^{***}
<i>P. scolymocephala</i>	71±7.71 ^a	14±3.49 ^d	24±3.64 ^{dc}	53±7.71 ^{abc}	11±3.49 ^d	23±3.64 ^d	59±7.71 ^a	11±3.49 ^d	30±3.64 ^{db}	53±7.71 ^{ab}	14 ±3.49 ^d	25±3.64 ^{dc}	6.489 ^{***}
<i>S. elegans</i>	91±5.91 ^a	8±7.55 ^e	35±5.66 ^{dc}	66±5.91 ^{abc}	49±7.55 ^{db}	44±5.66 ^{dc}	94±5.91 ^a	44±7.55 ^{dc}	34±5.66 ^{dec}	85±5.91 ^{ab}	22±7.55 ^{de}	19±5.66 ^{de}	19.593 ^{***}
<i>S. fasciflora</i>	41±8.95 ^{ad}	5±3.56 ^{fe}	144±4.23 ^{lbc}	44±8.95 ^{ac}	10±3.56 ^{fd}	20±4.23 ^{lbd}	35±8.95 ^{abcde}	6±3.56 ^f	18±4.23 ^{lbcd}	45±8.95 ^{ab}	8±3.56 ^{fd}	17±4.23 ^{lcd}	4.143 ^{**}
<i>T. punctatus</i>	54±7.73 ^{abc}	34±3.32 ^{dbc}	27±4.87 ^d	34±7.73 ^{dbc}	11±3.32 ^d	23±4.87 ^d	61±7.73 ^{ab}	21±3.32 ^d	33±4.87 ^{dc}	60±7.73 ^{ac}	19±3.32 ^d	31±4.87 ^{db}	3.625 ^{***}
<i>U. anthemoides</i>	99±0.88 ^a	98±1.33 ^a	33±6.31 ^a	100±0.88 ^a	96±1.33 ^a	84±6.31 ^a	99±0.88 ^a	89±1.33 ^a	34±6.31 ^b	99±0.88 ^a	94±1.33 ^a	49±6.31 ^b	22.705 ^{***}
<i>W. meriana</i>	86±2.76 ^a	12±2.02 ^d	48±6.00 ^b	91±2.76 ^a	96±2.02 ^a	53±6.00 ^b	96±2.76 ^a	96±2.02 ^a	34±6.00 ^{bc}	98±2.76 ^a	92±2.02 ^a	25±6.00 ^{dc}	36.695 ^{***}

Table 4.3: Effects of different desiccation treatments on seventeen indigenous CFSF species tested under shaded greenhouse conditions. Data are untransformed means \pm SE $n=4 \times 40$ seeds in percentages and results of repeated measures ANOVA values are shown (* $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$). The repeats of initial germination (pre), desiccation (dry) and rewatering maximum response in different treatments (i.e. control, 12 and 30 days).

	Control	12 days			30 days			
Species name	Control	Pre	Dry	Rew	Pre	Dry	Rew	ANOVA = $F_{(4,18)}$
<i>A. imbricata</i>	93.13 \pm 7.89 ^a	61.88 \pm 7.89 ^b	15.63 \pm 3.16 ^d	34.38 \pm 3.45 ^{dc}	48.3 \pm 7.89 ^{bc}	10.00 \pm 3.16 ^d	18.75 \pm 3.45 ^d	10.513***
<i>C. ciliata</i>	100 \pm 3.61 ^a	93.75 \pm 3.61 ^{ab}	28.13 \pm 5.01 ^d	68.75 \pm 2.73 ^c	100 \pm 3.61 ^a	47.50 \pm 5.01 ^d	75.00 \pm 2.73 ^{cb}	19.190***
<i>D. oppositifolia</i>	54.38 \pm 6.50 ^{ab}	65.00 \pm 6.50 ^a	25.00 \pm 4.78 ^c	36.88 \pm 4.20 ^{cb}	61.88 \pm 6.50 ^{ab}	18.75 \pm 4.78 ^c	22.50 \pm 4.20 ^c	15.160***
<i>E. mammosa</i>	75.00 \pm 7.42 ^{ac}	90.00 \pm 7.42 ^a	30.63 \pm 6.61 ^{fc}	49.38 \pm 5.89 ^{cbd}	78.75 \pm 7.42 ^{ab}	13.13 \pm 6.61 ^{fd}	36.88 \pm 5.89 ^{ec}	76.657***
<i>E. plumosa</i>	77.50 \pm 5.22 ^a	72.50 \pm 5.22 ^a	20.00 \pm 5.38 ^{gcf}	43.13 \pm 6.58 ^{dbe}	51.88 \pm 5.22 ^{abc}	11.25 \pm 5.38 ^{ge}	28.75 \pm 6.58 ^{df}	22.778***
<i>L. salignum</i>	68.13 \pm 10.78 ^{abd}	73.13 \pm 10.78 ^{ac}	20.00 \pm 8.01 ^{de}	34.38 \pm 8.78 ^{bde}	87.50 \pm 10.78 ^{ab}	8.75 \pm 8.01 ^e	34.38 \pm 8.78 ^{dc}	42.794***
<i>M. densa</i>	56.25 \pm 7.08 ^{ab}	88.13 \pm 7.08 ^a	23.13 \pm 4.96 ^{cd}	36.25 \pm 5.20 ^{cdb}	79.38 \pm 7.08 ^a	10.00 \pm 4.96 ^d	40.00 \pm 5.20 ^{cb}	40.511***
<i>P. corymbosa</i>	96.25 \pm 8.80 ^a	68.75 \pm 8.80 ^a	17.50 \pm 3.53 ^b	22.50 \pm 4.64 ^b	85.00 \pm 8.80 ^a	20.63 \pm 3.53 ^b	26.88 \pm 4.64 ^b	12.228***
<i>P. cephalantha</i>	55.00 \pm 9.41 ^{abe}	78.13 \pm 9.41 ^{ad}	30.63 \pm 8.31 ^{ec}	52.50 \pm 8.36 ^{bf}	81.25 \pm 9.41 ^{abc}	21.25 \pm 8.31 ^{ef}	36.88 \pm 8.36 ^{edf}	17.304***
<i>P. repens</i>	89.38 \pm 8.20 ^a	65.00 \pm 8.20 ^{abc}	13.75 \pm 5.63 ^{ge}	32.50 \pm 4.85 ^{df}	78.75 \pm 8.20 ^a	28.13 \pm 5.63 ^{gcf}	45.63 \pm 4.85 ^{dbe}	22.630***
<i>P. scolymocephala</i>	75.00 \pm 7.19 ^{ab}	86.25 \pm 7.19 ^a	19.38 \pm 6.06 ^{ed}	48.75 \pm 6.09 ^{cb}	38.13 \pm 7.19 ^{cbd}	10.63 \pm 6.06 ^e	21.88 \pm 6.09 ^{ec}	26.840***
<i>S. elegans</i>	100 \pm 3.30 ^{ab}	88.13 \pm 3.30 ^{abc}	29.38 \pm 4.52 ^e	51.25 \pm 87.79 ^{de}	100 \pm 3.30 ^a	61.25 \pm 4.52 ^{dc}	74.38 \pm 7.79 ^{bd}	11.836***
<i>S. fasciflora</i>	48.75 \pm 9.38 ^{ad}	60.63 \pm 9.38 ^{ac}	11.25 \pm 7.34 ^d	25.63 \pm 7.34 ^{db}	63.13 \pm 9.38 ^{ab}	25.00 \pm 7.34 ^{dc}	41.25 \pm 7.69 ^{dc}	13.769***
<i>T. punctatus</i>	58.13 \pm 8.04 ^{abc}	50.63 \pm 8.04 ^{ac}	11.25 \pm 6.57 ^d	22.50 \pm 7.56 ^{db}	76.88 \pm 8.04 ^a	36.88 \pm 6.57 ^{dc}	60.63 \pm 7.56 ^{ab}	12.834***
<i>U. anthemoides</i>	100 \pm 4.03 ^a	93.75 \pm 4.03 ^a	20.00 \pm 3.70 ^e	51.88 \pm 4.38 ^{cd}	96.88 \pm 4.03 ^{ab}	39.38 \pm 3.70 ^{ed}	72.50 \pm 4.38 ^{cb}	17.923***
<i>W. multiflora</i>	100 \pm 5.26 ^a	91.88 \pm 5.26 ^a	21.25 \pm 3.82 ^d	44.38 \pm 3.62 ^{bc}	80.63 \pm 5.26 ^a	21.25 \pm 3.82 ^{dc}	54.38 \pm 3.62 ^b	46.872***
<i>W. meriana</i>	89.38 \pm 5.18 ^{ab}	86.25 \pm 5.18 ^{ac}	45.00 \pm 6.52 ^{de}	54.38 \pm 7.66 ^{deb}	93.13 \pm 5.18 ^a	27.50 \pm 6.52 ^e	55.63 \pm 7.66 ^{dbc}	25.321***

Figures

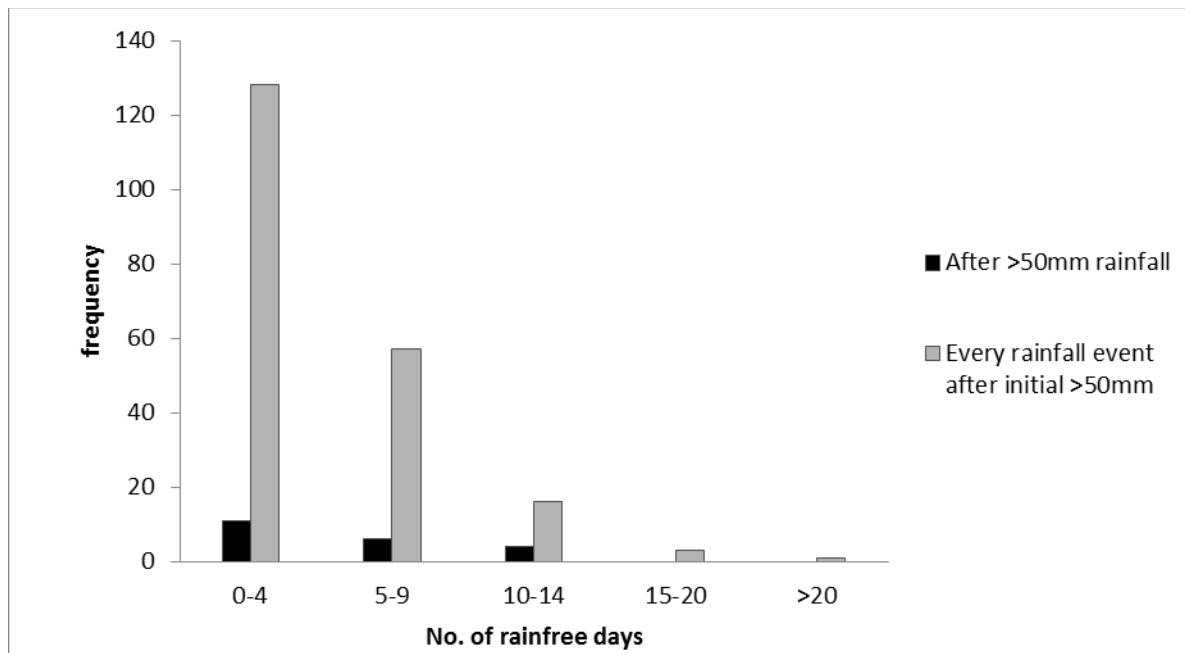
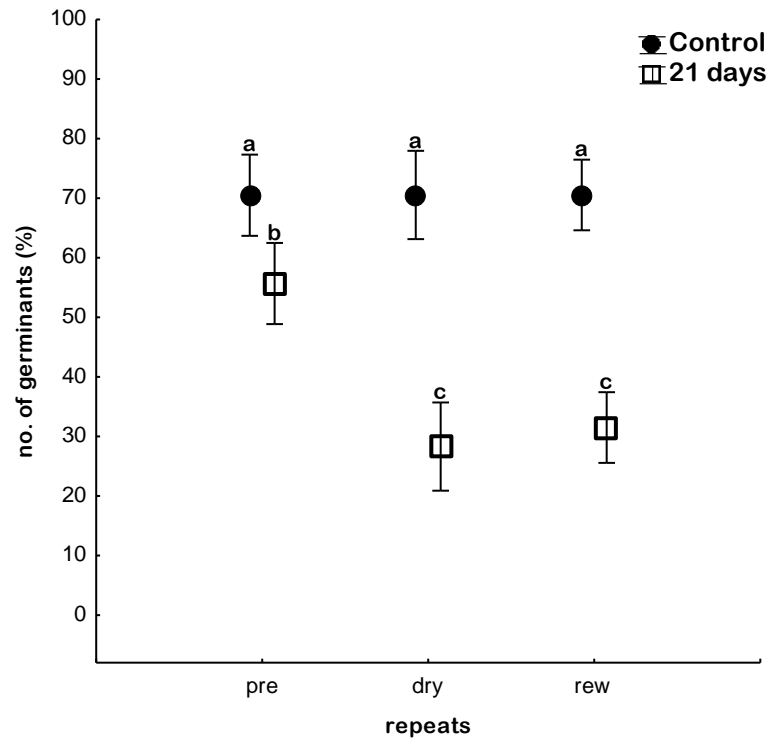
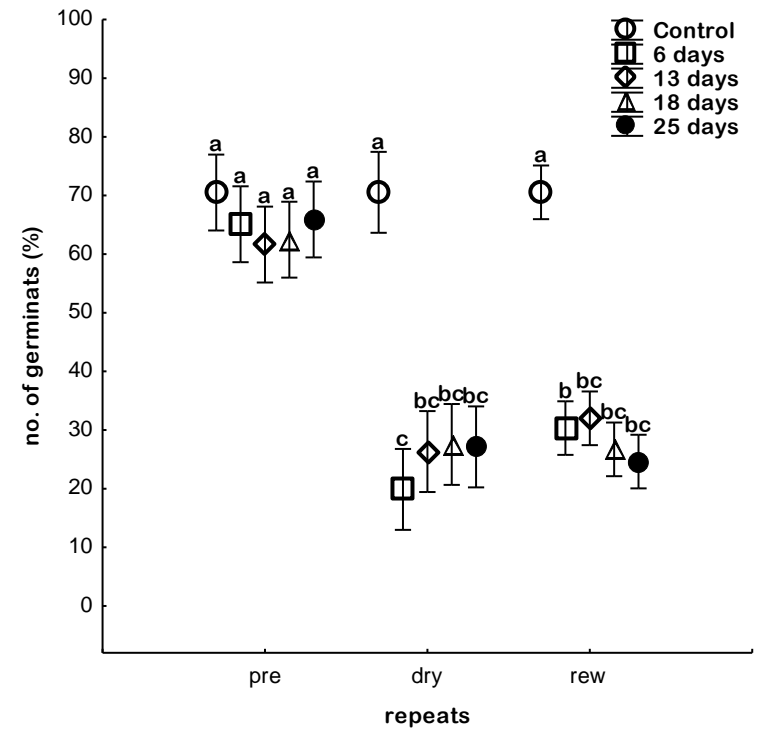


Figure 4.1: A 20-year frequency graph showing rainfall pattern summary (April to September) for number of rain-free days after a rainfall event and/or after an initial rainfall event >50mm. Data were obtained Cape Town Weather Observation station records from 1995 to 2014.



a) Pre-germination drying-down of control and 21 days.



b) Post-germination drying-down in Control, 6, 13, 18 and 25 days.

Figure 4.2: Least squared means plots (mean % germination \pm SE) showing overall effects on survived germinants of a) Pre- and b) Post-germination desiccation experiment conducted in the growth chamber using CFSF species with the repeats of initial germination (pre), desiccation (dry) and rewatering maximum response in different treatments (i.e. control, 21,6,13,18 and 25 days).

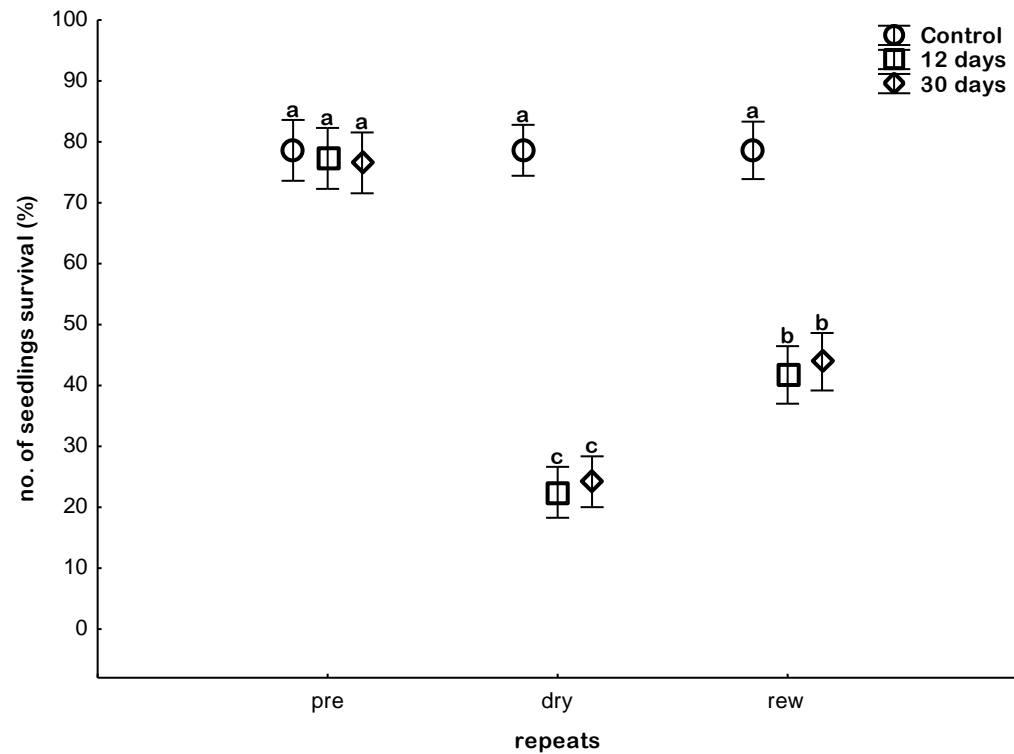
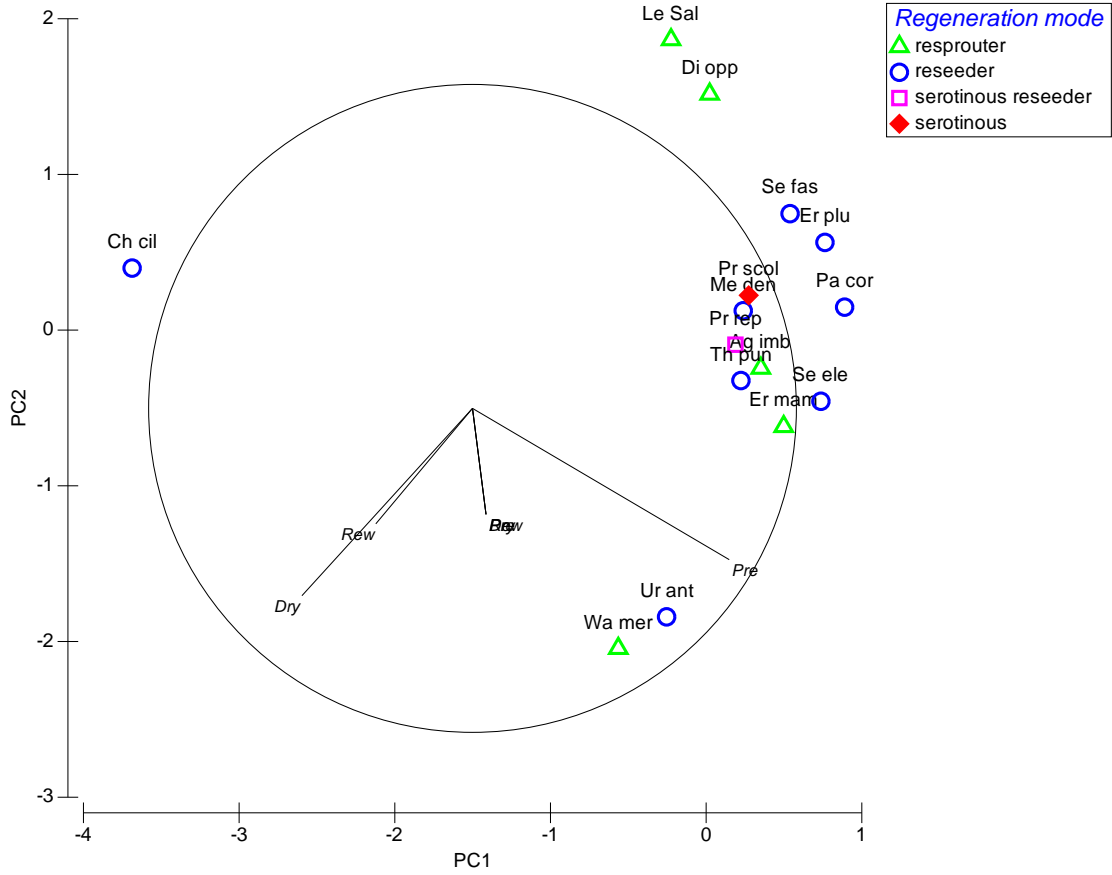
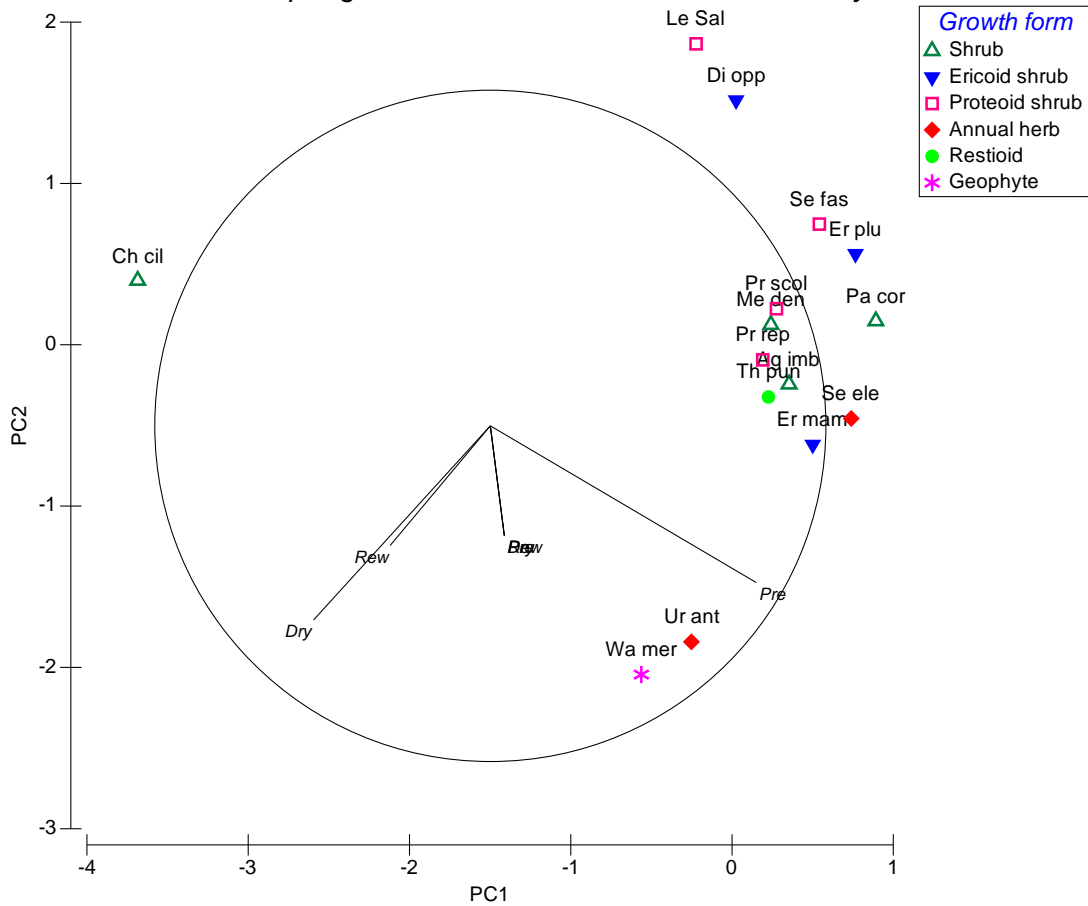


Figure 4.3: Least squared means (mean % germination \pm SE) plot showing overall effects of seedling emergence of desiccation experiment conducted in the greenhouse using CFSF species with repeats of initial germination (pre), desiccation (dry) and rewatering maximum response in different treatments (i.e. control, 12, and 30 days).

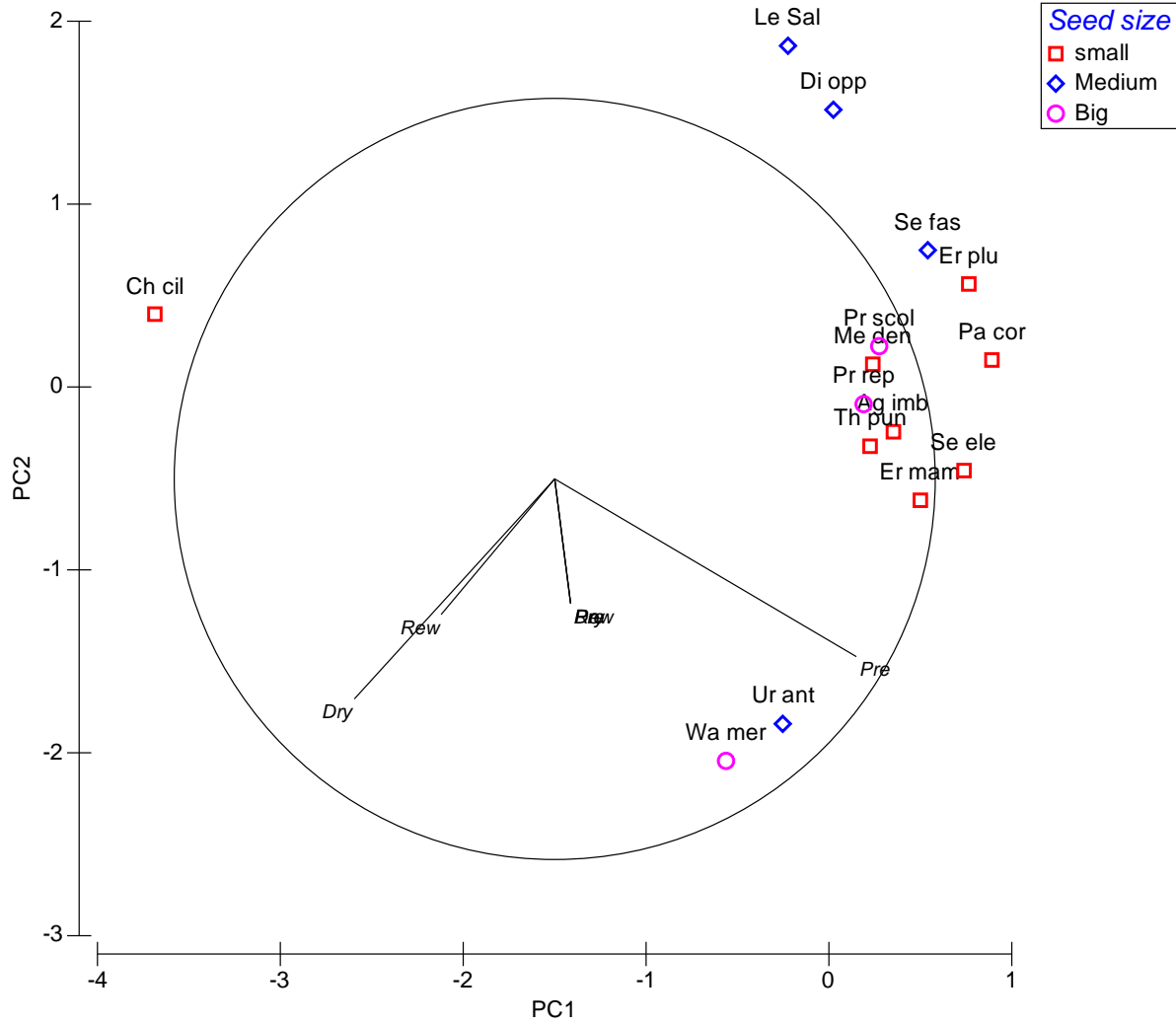
Seed pre-germination desiccation Control & 21 days



Seed pre-germination desiccation Control & 21 days

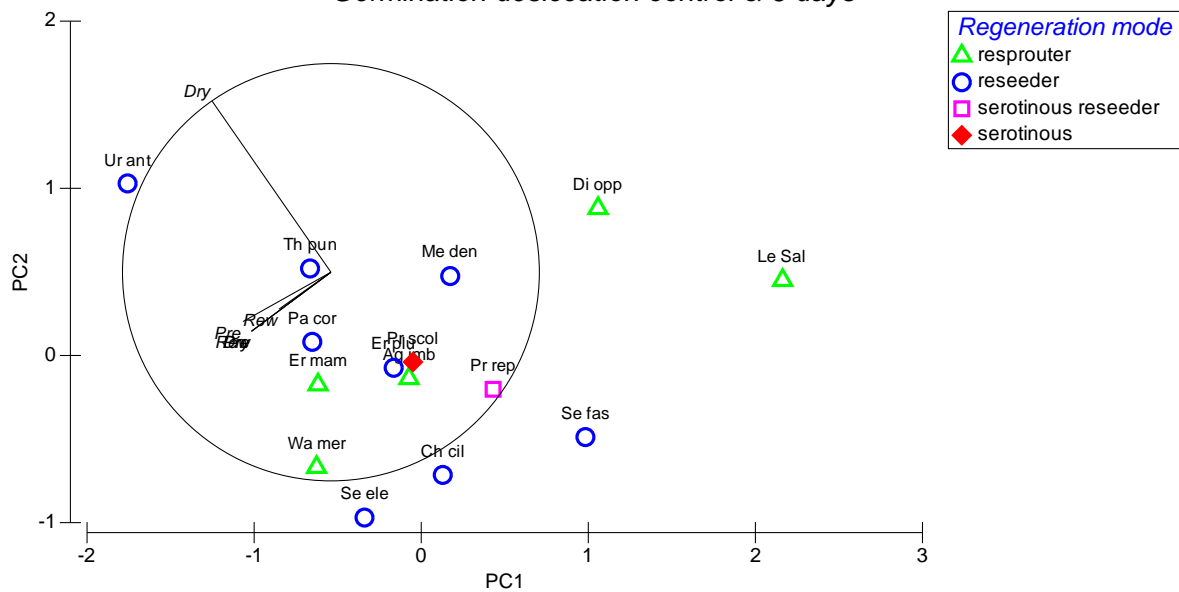


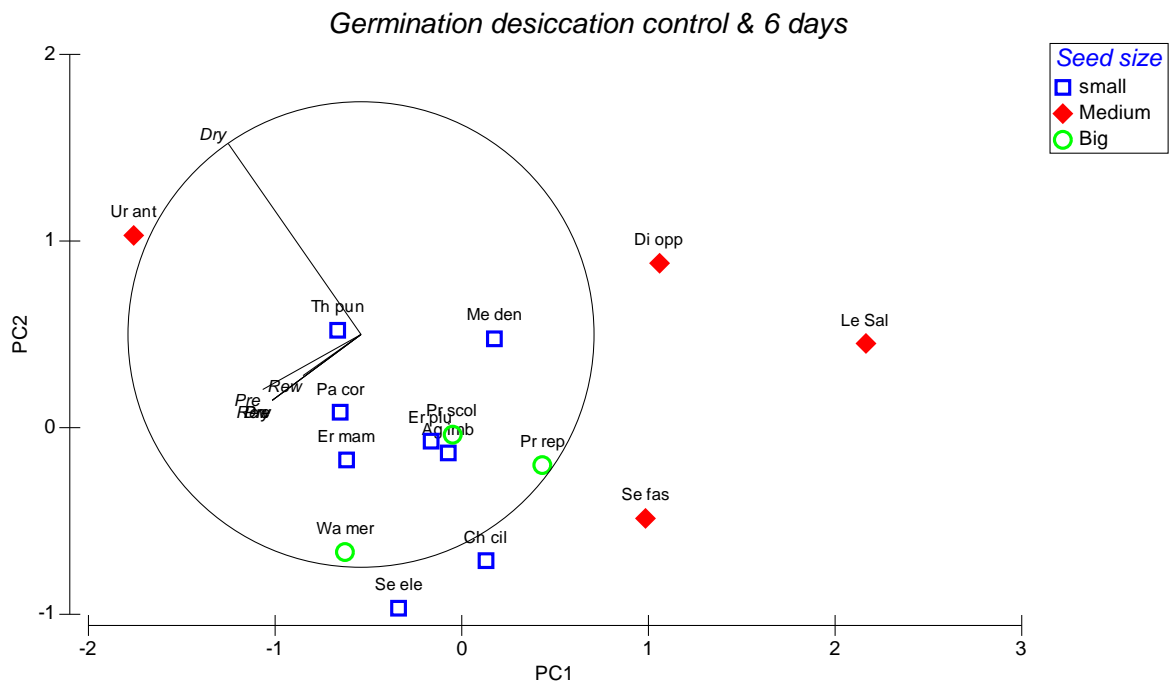
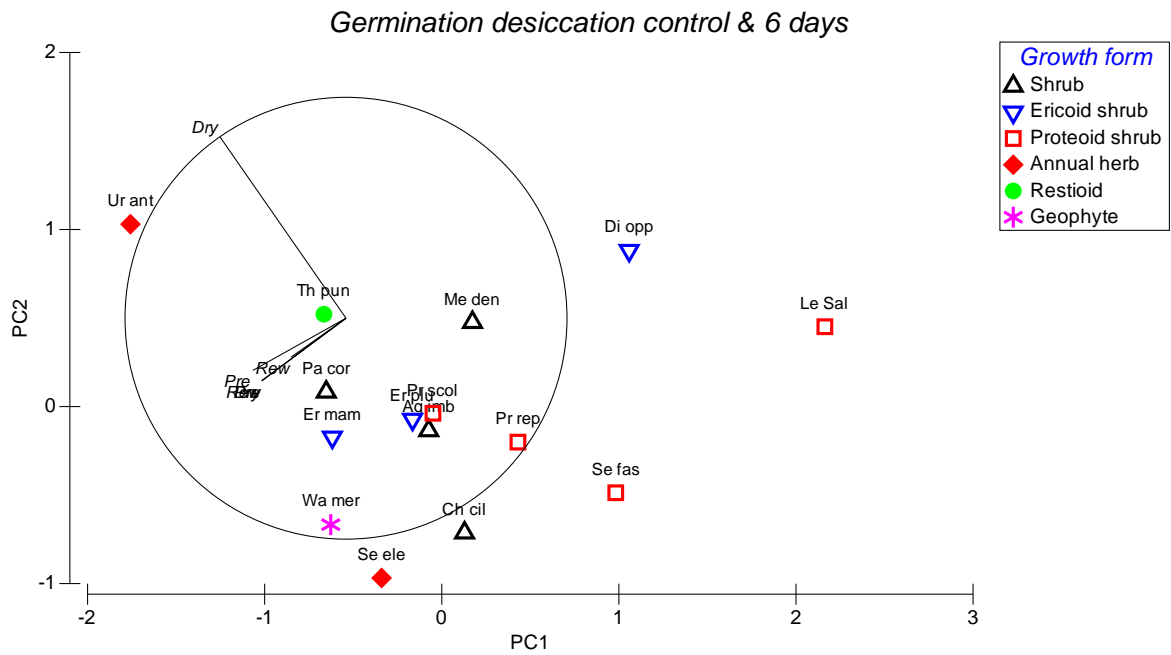
Seed pre-germination desiccation Control & 21 days



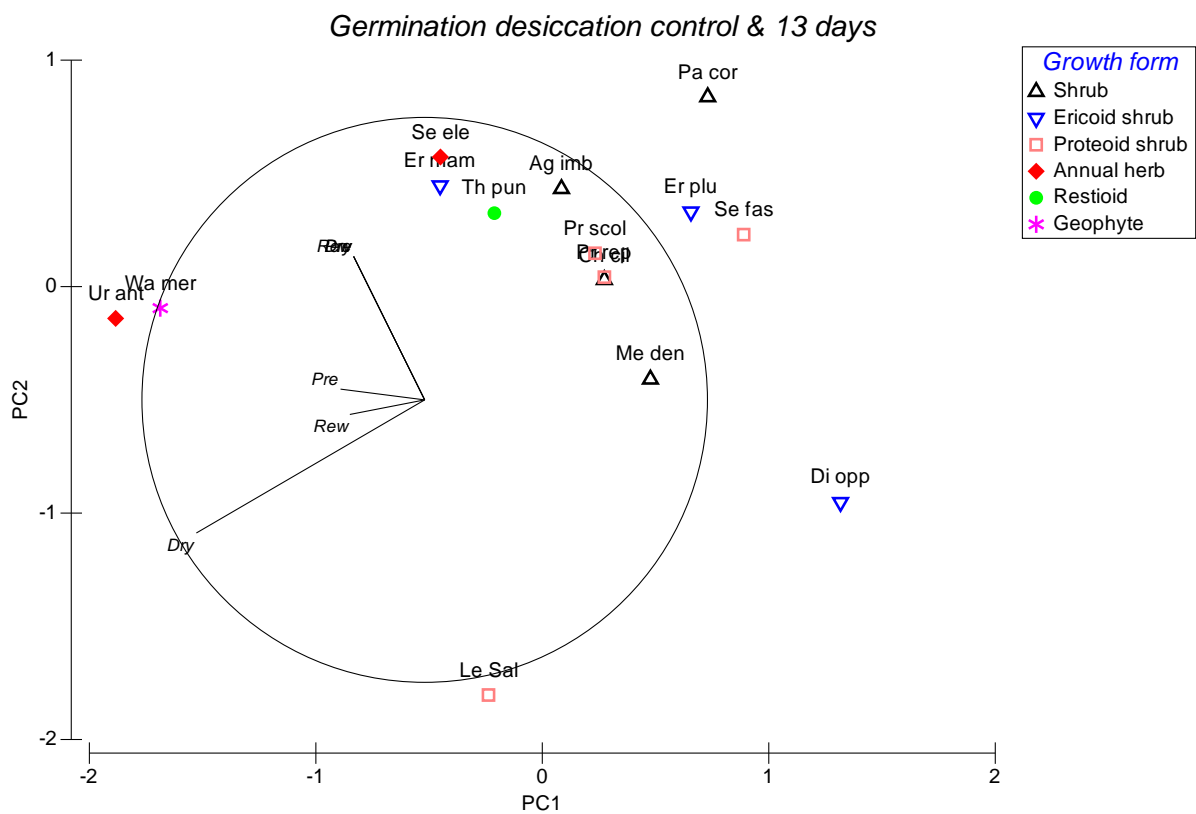
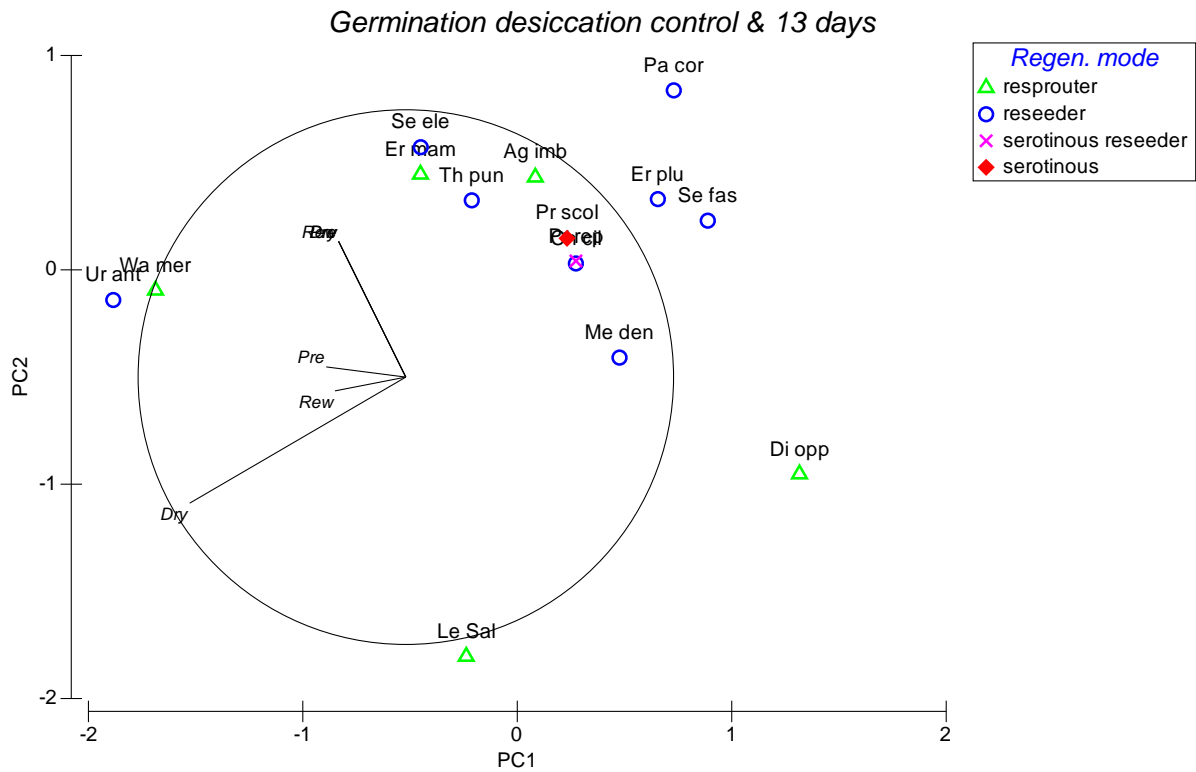
a) Regeneration mode, growth form, and seed size for control and 21 days germination desiccation.

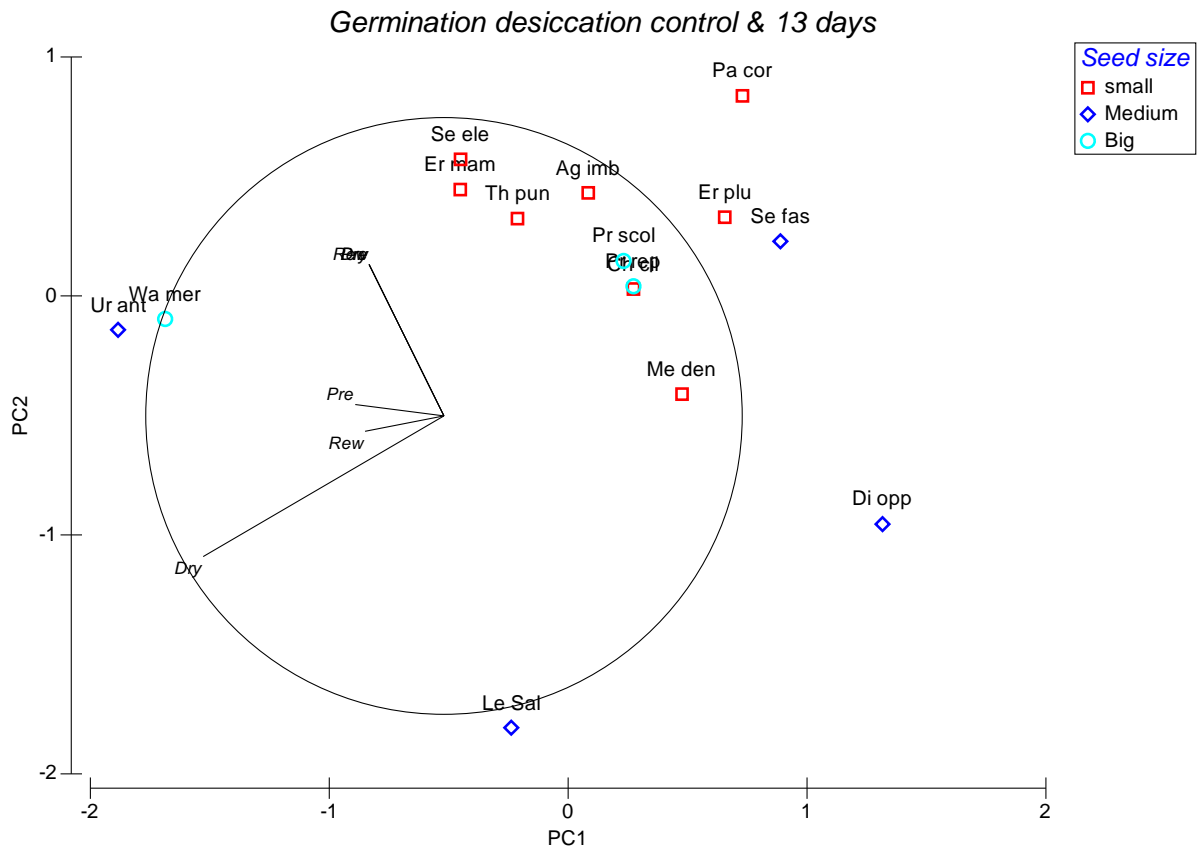
Germination desiccation control & 6 days



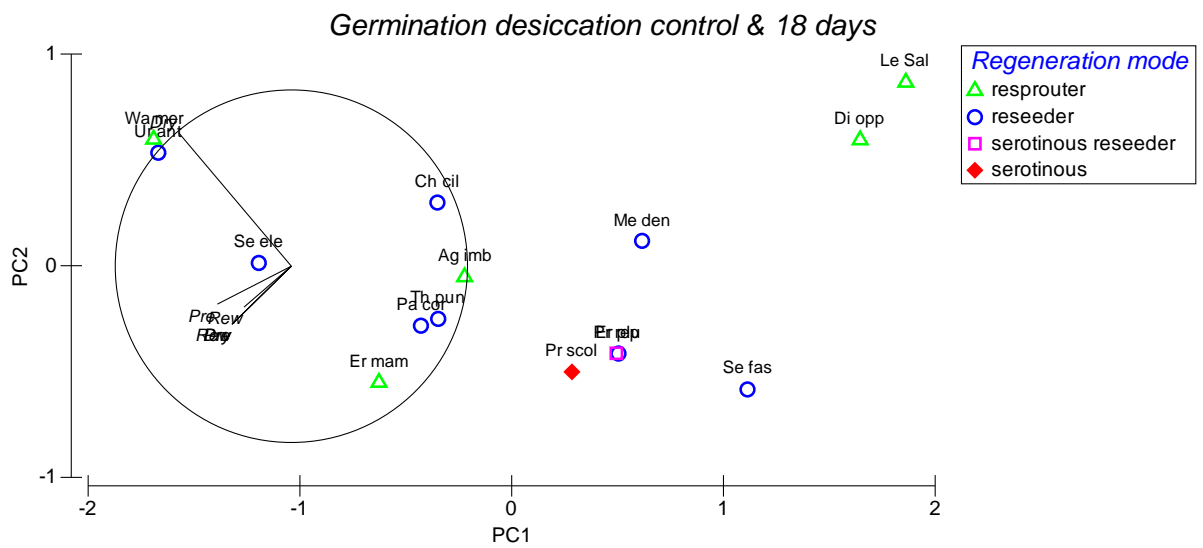


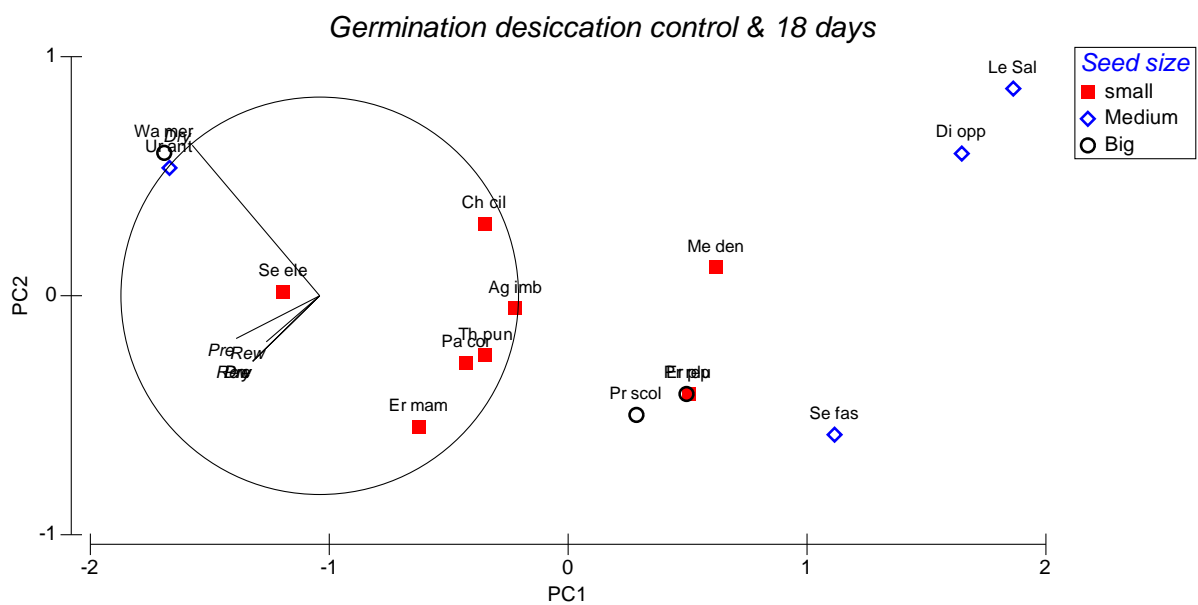
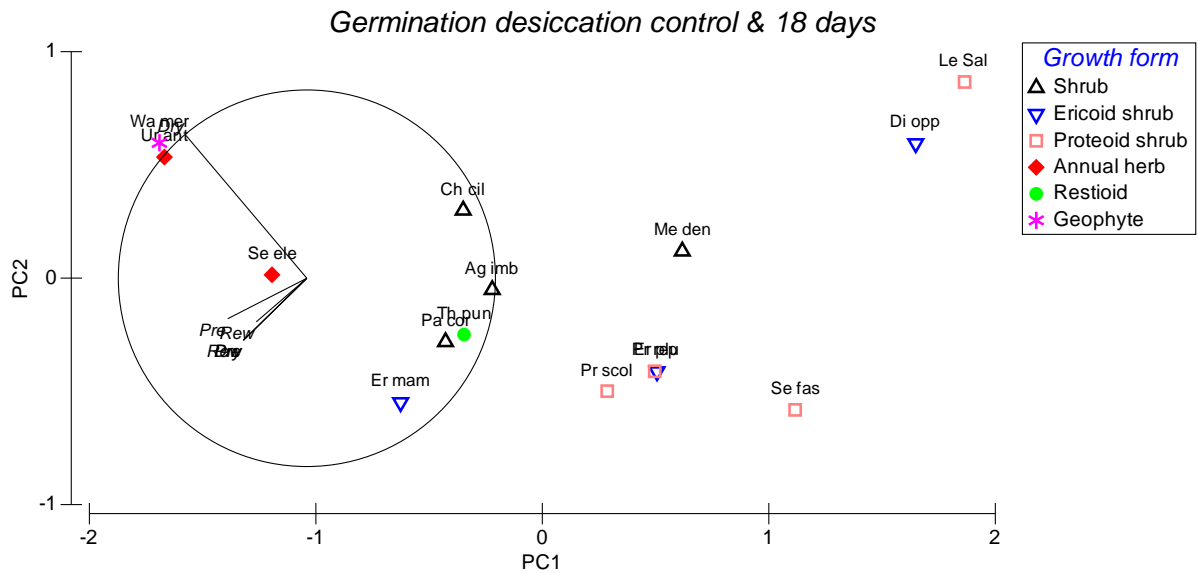
b) PCA plots for regeneration mode, growth form, and seed size of seed germinants drying-down for Control and 6 days.





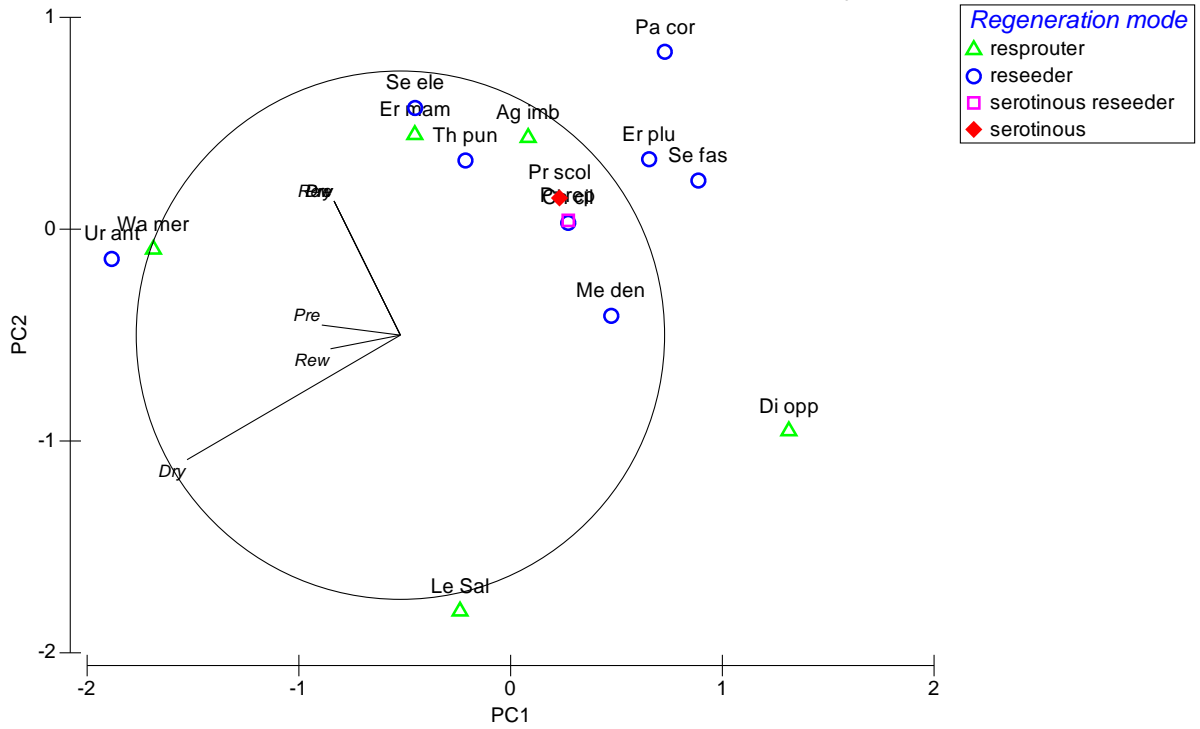
c) PCA plots for regeneration mode, growth form, and seed size of seed germinants drying-down for Control and 13 days.



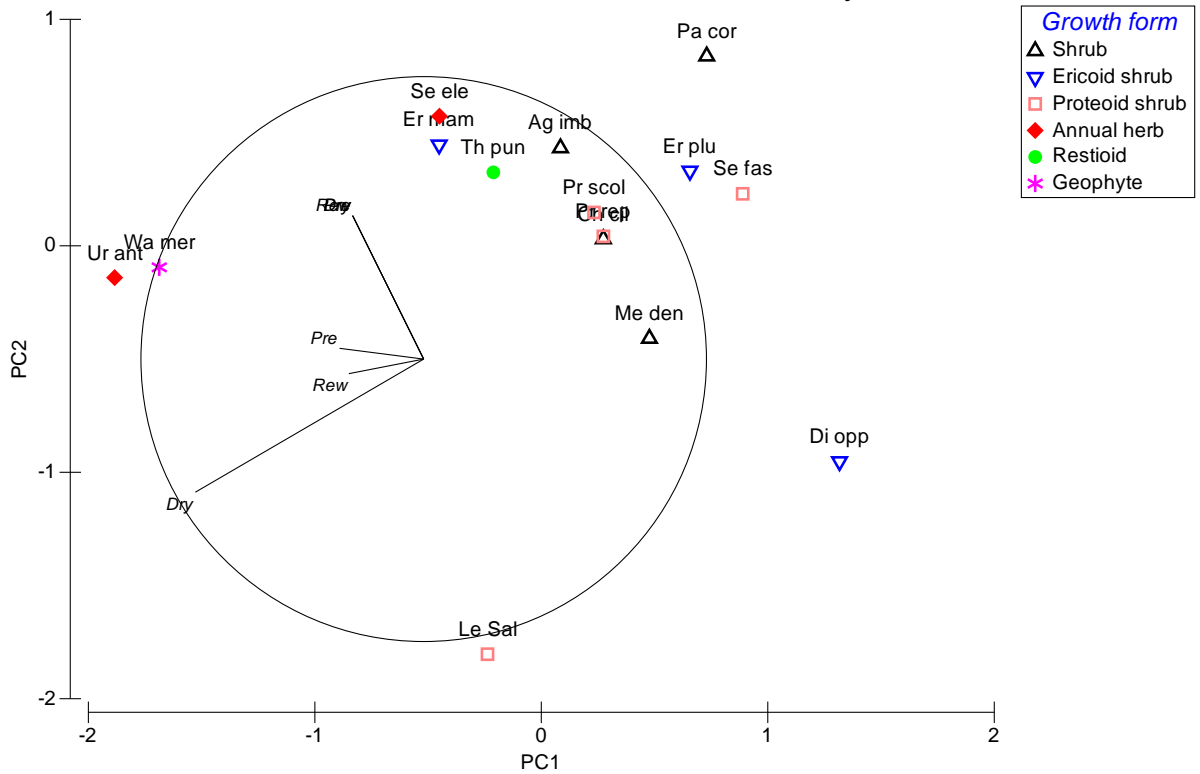


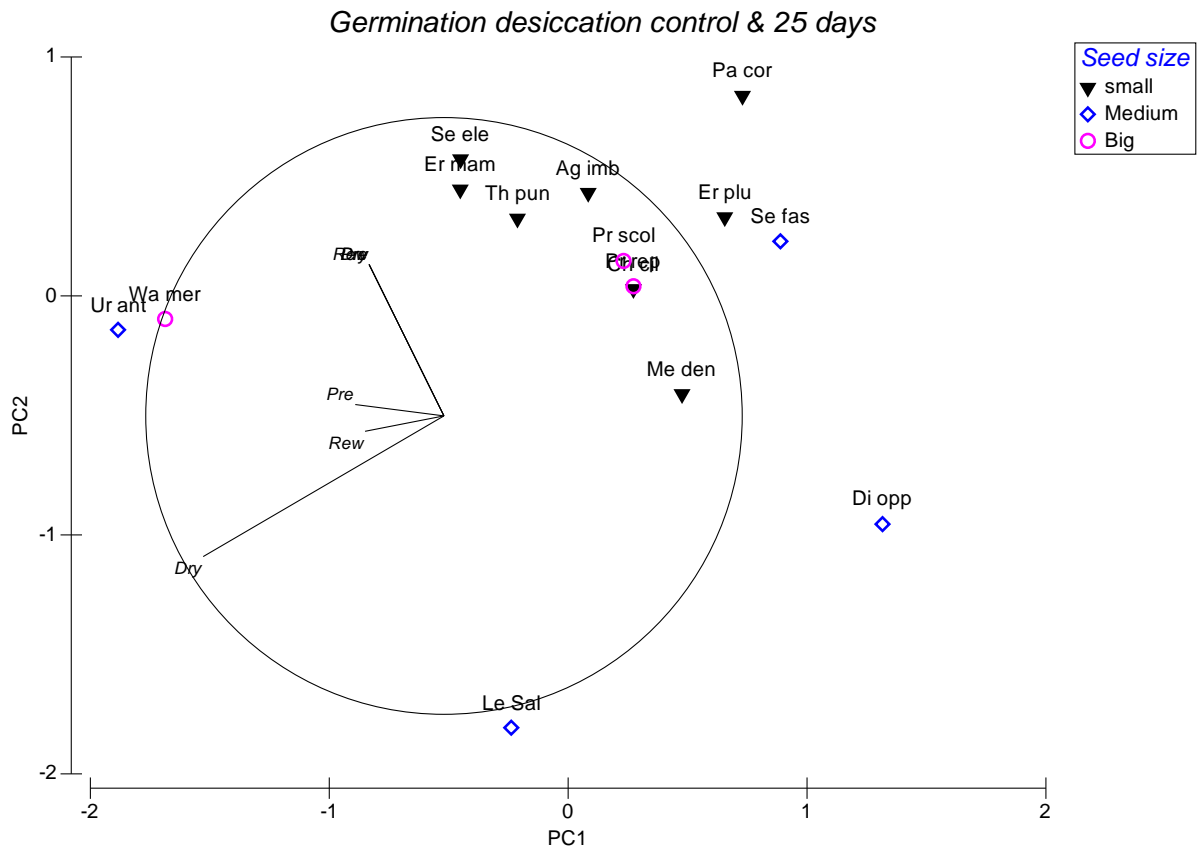
d) PCA plots for regeneration mode, growth form, and seed size of seed germinants drying-down for Control and 18 days.

Germination desiccation control & 25 days



Germination desiccation control & 25 days





e) PCA plots for regeneration mode, growth form, and seed size of seed germinants drying-down for Control and 25 days.

Figure 4.4: Principal Component analysis (PCA) for regeneration mode, growth form, and seed size of seed germinants drying-down for Pre- and Post-germination treatment for 15 CFSF species showing axes of variation in Pre (initial germination), dry (desiccation), rew (rewatering), in a) 21, b) 6, c) 13, d) 18 and e) 25 days.

CHAPTER 5: SYNTHESIS

5.1. SUMMARY OF FINDINGS AND RECOMMENDATIONS FOR LARGE-SCALE RESTORATION OF CFSF

This study was designed to find answers for limited germination of re-introduced Cape Flats Sand Fynbos (CFSF) vegetation species after removal of invasive Australian *Acacia* species and to give recommendation for large-scale restoration. Poor seedling emergence can either be due to post-germination factors, such as drought following germination or due to a lack of appropriate pre-germination cues that overcome dormancy. The first part of my study focused on pre-germination factors (i.e., testing different pre-germination cues) whereas the second part focused on post-germination factors (i.e., drought as a reason for poor seedling establishment).

The aim of this research is to provide management recommendations for seed pre-germination treatments in active restoration of lowland sand fynbos. The pre-germination experiment showed that smoke water treatment resulted in positive germination results in majority of species selected. However, some species had a positive response to the combination of heat and smoke treatment, which indicates that in some cases smoke alone, is not the optimal cue. Other species had a positive response to scarification and a combination of scarification and smoke. Heat did not enhance germination of some species, which could mean that the embryo of the seed was damaged by heat or else it had no effect. Seeds of species that did not respond to any treatment still did germinate in treatments (albeit low percentages, similar to the control). In summary, this study shows that despite a few species non-responsiveness to treatment, majority of species responded to a variety of pre-germination treatments, which I consider crucial for enhancing CFSF restoration success. The lack of pre-germination treatments applied to seed in earlier restoration efforts on this vegetation type is a potential reason for a lack of germination of several indigenous fynbos plant species.

This study only tested a handful of the many hundreds of CFSF species, but was a representative sample of the main growth form guilds and therefore gives insight on potential species to use in large-scale restoration and the required pre-germination treatments to use. In light of this study, restoration efforts that use direct seeding should first study targeted species' dormancy-breaking requirements (these are species-specific) prior to sowing seeds.

As part of the preparation process, seed viability should be determined. For example, a cut test should be used in the field while collecting seeds and in the laboratory, and include a tetrazolium test after collection. This helps seed collectors to estimate the proportion of damaged, empty, and mature seeds and therefore to estimate quantities needed. Care should be taken to store seeds in a cool, dry, insect free place since exposure to inappropriate conditions can damage the collection (Gosling, 2003; Royal Botanical Gardens KEW, 2008) an increase in temperature and moisture may promote fungal development (Roberts, 1972) and insect development in seeds (Christensen, 1972). It is also recommended that collection of seeds be performed when they are naturally dispersing wherever possible, because that is the time when most seeds will be mature (and this will account for the cut test, which is not practical in field for the small seeded species).

The smoke application experiment showed that majority of CFSF species require smoke pre-germination treatment, be it either pre-germination smoke treatment of soil, seed, or combination of both soil and seed in order to enhance germination. This is because the responses to gaseous smoke pre-germination treatment were species-specific. From observation, the most reliable and efficient outcome can be achieved when seeds and soil are smoke treated prior to their sowing when used in restoration efforts. Application of smoke in the field can be done by smoke water (using automated sprayers) or aerosol smoke (albeit area for treatment will be limited using this method).

Smoke treatment, in particular, can effectively break dormancy or promote germination by directly penetrating the seed or indirectly by adsorption onto soil particles that later releases chemical (Keeley and Fotheringham, 1998). Therefore, as already highlight, pre-smoking of both seeds and soil should be performed to enhance germination. Seeds should be pre-smoked even if restoration efforts include controlled burns, this is to make-up for the washed out smoke chemicals during winter rainfall. It is recommended to sow to seeds earlier after fire otherwise smoke chemicals in soil may have dissipated (Musil and de Witt, 1990; Keeley and Fotheringham, 1998).

The desiccation tolerance of seeds in both greenhouse and growth chamber experiments varied according to species. Drying-down of seeds before germination had no effect on germination in 13 species, whereas two species survived drying-down with lower germinants after rewatering. Some species survived drying-down after rewatering wherein the number of germinants increased, whilst other species' germinants decreased after rewatering. These results suggest that majority of CFSF species are desiccation tolerant and there was no pattern in regeneration mode, seed size and growth form in relation to desiccation. This could mean that drought is not a major cause of poor germination in the field after alien clearing and sowing and that there could be other environmental factors involved.

5.2. SUGGESTIONS FOR FURTHER RESEARCH

In this study, fynbos species were used as combustion biomass; however, the actual field combustion biomass in areas where *Acacias* have been removed is usually a combination of fynbos and *Acacia saligna*. It may be worth investigating volatile chemical compounds of burnt *Acacia saligna* that might have different effects than fynbos material on seed germination of indigenous fynbos species in the field (Keeley and Pizzorno, 1986). This is because studies show that unfavourable shrub-dominated environments (Koller, 1972; Angevine and Chabot, 1979; Keeley, 1991) release compounds best described as 'infochemicals' (Smith and van Staden, 1995) that inhibit germination.

A field experiment run in parallel with a greenhouse study will be necessary to follow-up on the findings of this study for the pre-germination cues. This is to ensure that confounding factors such as interactions with other cues, time since fire and season that could contribute to the response or lack of response of species are accounted for. For future research it is therefore recommended that granivory and herbivory needs to be minimised to ensure success in the field. Thomas and Davis, (1990) concluded that seedling ability to survive drought is of much importance than initial seedling numbers, therefore there is a need for further research on post-fire mortality and water relations of the CFSF species in the field. This is in comparison with a study by Musil (1993) who found that plants from infertile sites like *Protea repens* usually have low growth rates, low nutrient absorption rates and they do not respond to nutrient availability. Hence, further research is required to look at the effects of competition for moisture regime by indigenous fynbos plants at different levels.

The heat pulse experiment, applied by exposing seeds in an oven for 5 minutes in 100°C, might have damaged seeds in some species. This treatment should therefore only be applied to species for which a positive response to heat treatment has been established. Further research is needed on optimal heat pulse intensity and duration, in order to maximize germination responses.

Overall, the species that did not respond to any of the germination cues tested require further research (although most of them were not inhibited by smoke water pre-germination treatment).

5.3. CONCLUSIONS

It is important to conduct viability and germination trials prior to restoration, but in addition, this study suggests that the lack of pre-germination treatments is one cause of low indigenous fynbos species germination following removal of Australian *Acacia* species. Seed desiccation does not; however, appear to be a likely cause for observed low indigenous species establishment in the field. For effective restoration, efforts involving re-seeding, pre-germination treatments are essential, but as shown by the large variation in species responses treatments are likely to be species-specific.

REFERENCES

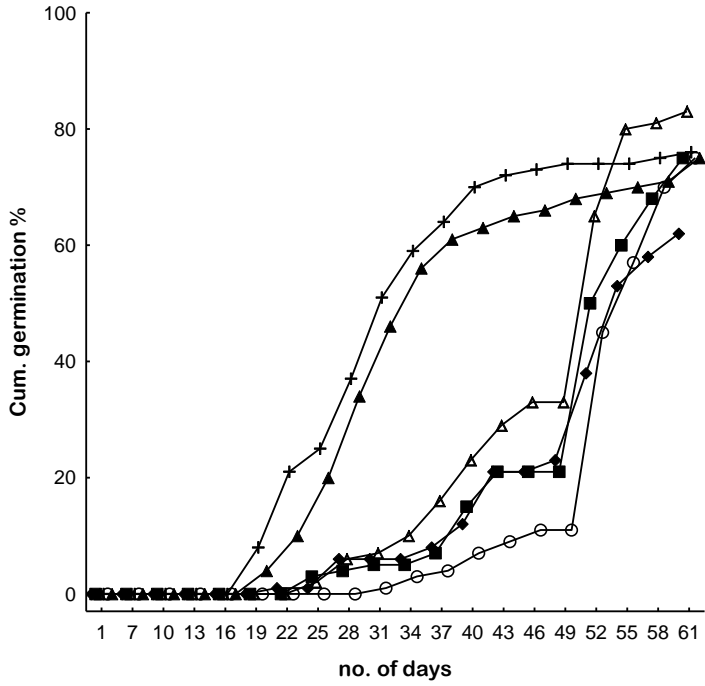
- Angevine, M.W., Chabot, B.F., 1979. Seed germination syndromes in higher plants. In: Solbrig, O.T., Jain, S., Johnson, G.G., Raven, P.H. (Eds.). Topics in Plant Population Biology. Columbia University Press, New York. pp. 188-206.
- Christensen, C.M., 1972. Micro flora and seed deterioration. In: Roberts, E.H. (Ed.). Viability of seeds. Chapman and Hall, London. pp. 59-93.
- Gosling, P.G., 2003. Viability testing. In: Smith, R.D., Dickie, J.B., Linington, S.L., Pritchard, H.W., Probert, R.J., (Eds.). Seed conservation turning science into practice. Royal Botanical Gardens, Kew. pp 445–481.
- Jones, R.M., Roux, E.R., Warren, J.M., 1963. Studies in the autecology of Australian acacias in South Africa. 4. The production of toxic substances by *Acacia cyclops* and *Acacia cyanophylla* and their possible ecological significance. South African Journal of Science 59, 295-296.
- Keeley, J.E., 1991. Seed germination and life history syndromes in the California chaparral. Botanical Review 57, 81-116.
- Keeley, J.E., Fotheringham, C.J., 1998. Smoke-induced seed germination in California chaparral.
- Keeley, S.C., Pizzorno, M., 1986. Charred wood stimulated germination of two fire-following herbs of the California chaparral and the role of hemicellulose. American Journal of Botany 73, 1289-1297.
- Koller, D., 1972. Environmental control of seed germination. In: Kozlowski, T.T. (Ed.). Seed Biology, Vol. II. Academic Press, New York. pp. 1-107.
- Moore, R.P., 1985. Handbook on tetrazolium testing. Tetrazolium Committee of the International Seed Testing Association. The International Seed Testing Association, Zurich, Switzerland. pp. 9-19.
- Musil, C.F., De Witt, D.M., 1990. Post-fire regeneration in a sand plain lowland fynbos community. South African Journal of Botany 56(2), 167-184.
- Roberts, E.H., 1972. Storage environment and the control of viability. In: Roberts, E.H. (Ed.). Viability of seeds. Chapman and Hall, London, UK. pp. 14-58.

Royal Botanical Gardens KEW, 2008. Seed Information Database (SID), Version 7.1.
Available at <http://data.kew.org/sid/> (May 2014).

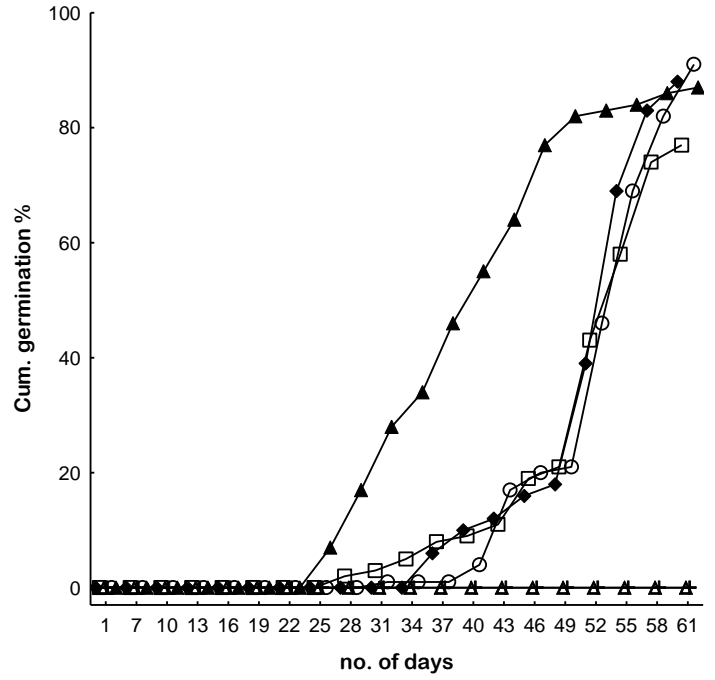
Smith, M.T., van Staden, J., 1995. Infochemicals: the seed-fungus-root continuum.
Environmental and Experimental Biology 35, 113-123.

APPENDICES

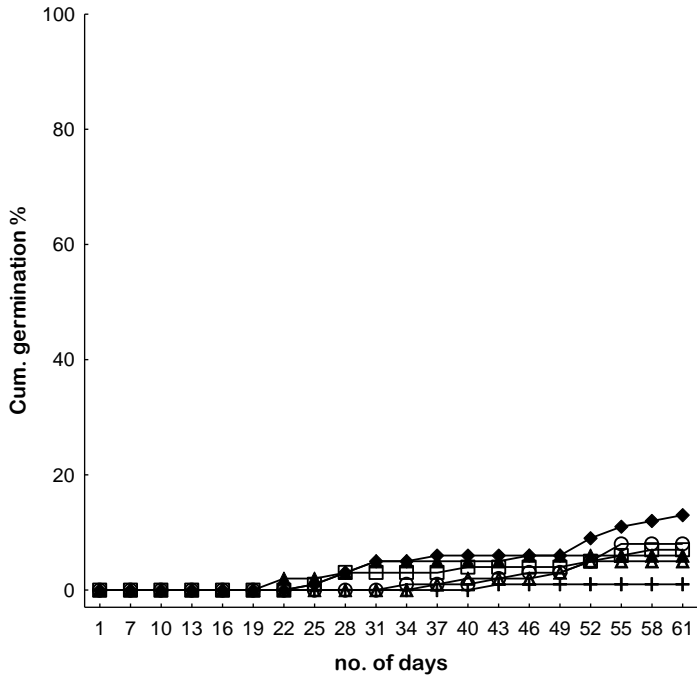
Appendix A.1.



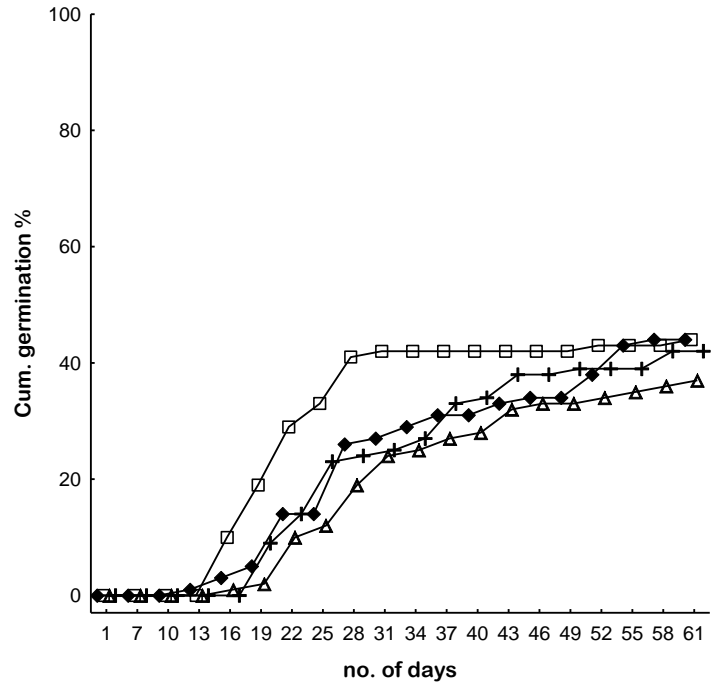
a) *Agathosma imbricata*



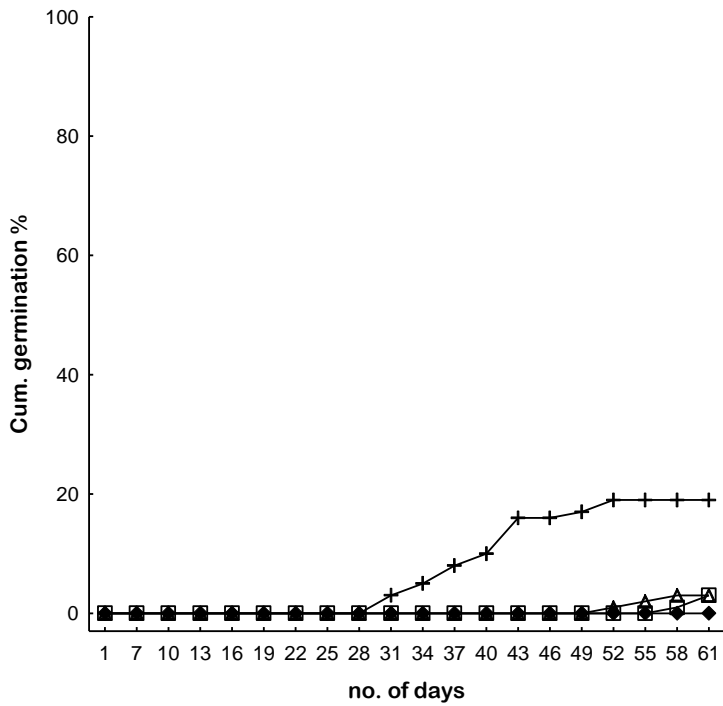
b) *Babiana villosula*



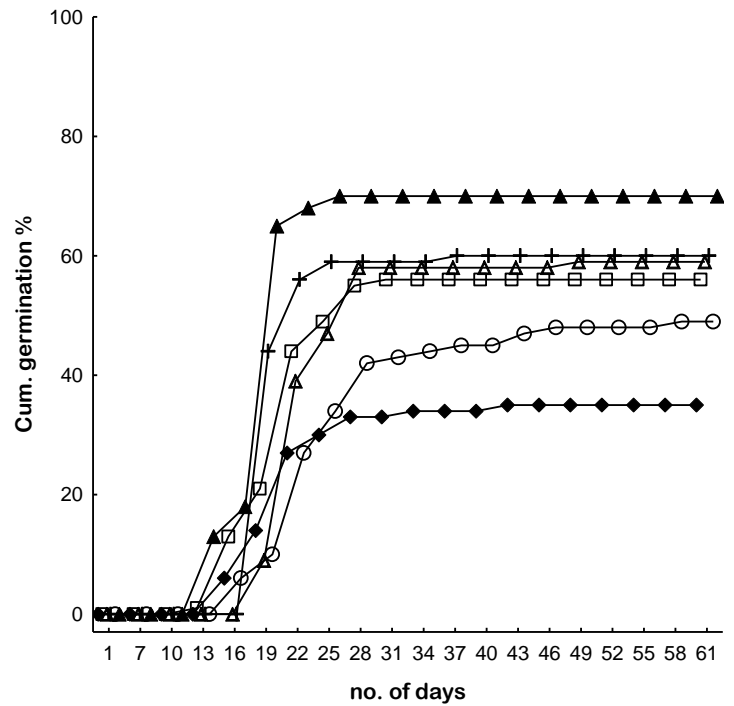
c) *Diosma oppositifolia*



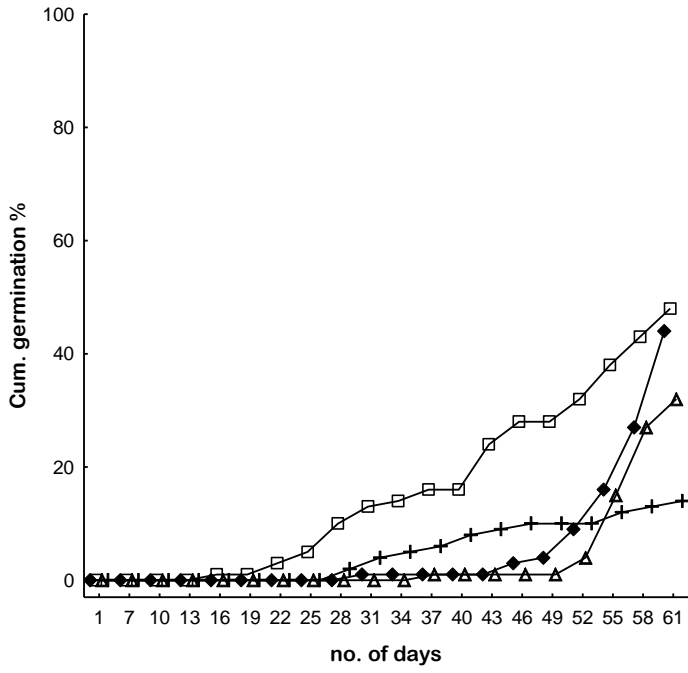
d) *Erica mammosa*



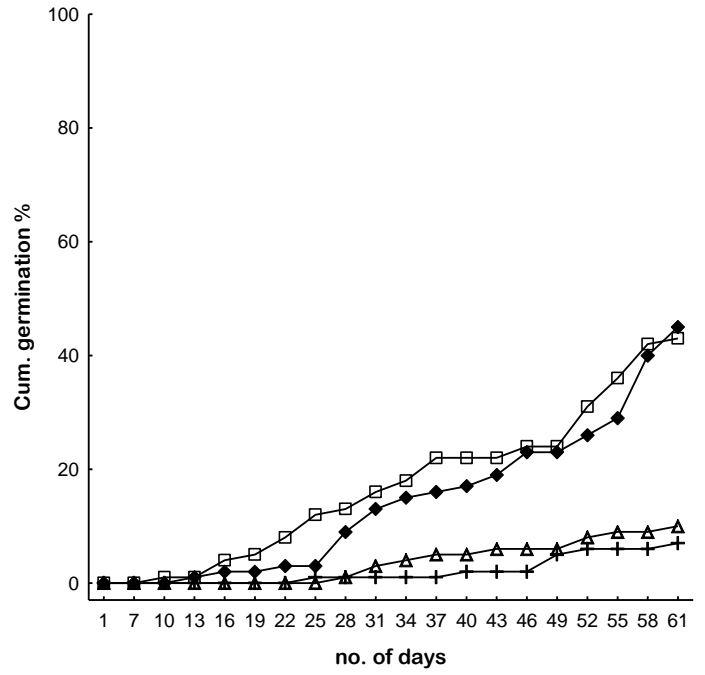
e) *Erica plumosa*



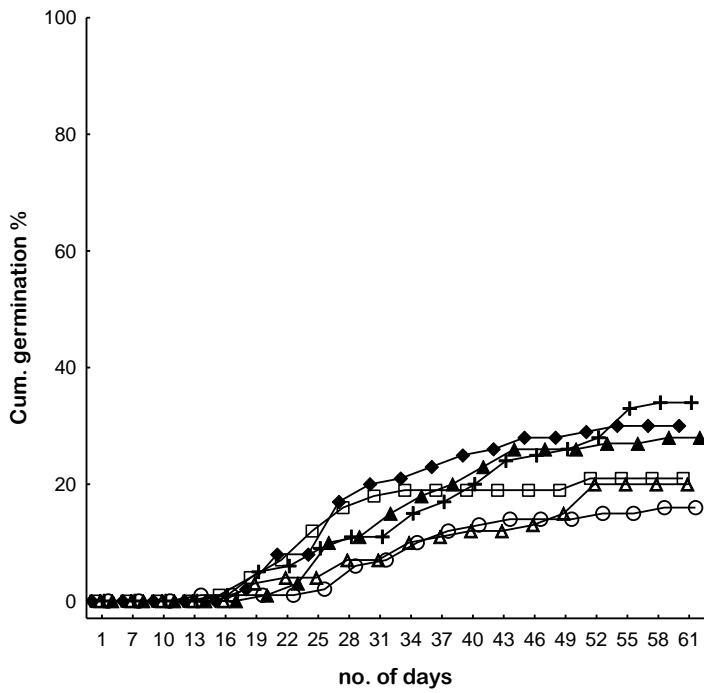
f) *Leucadendron salignum*



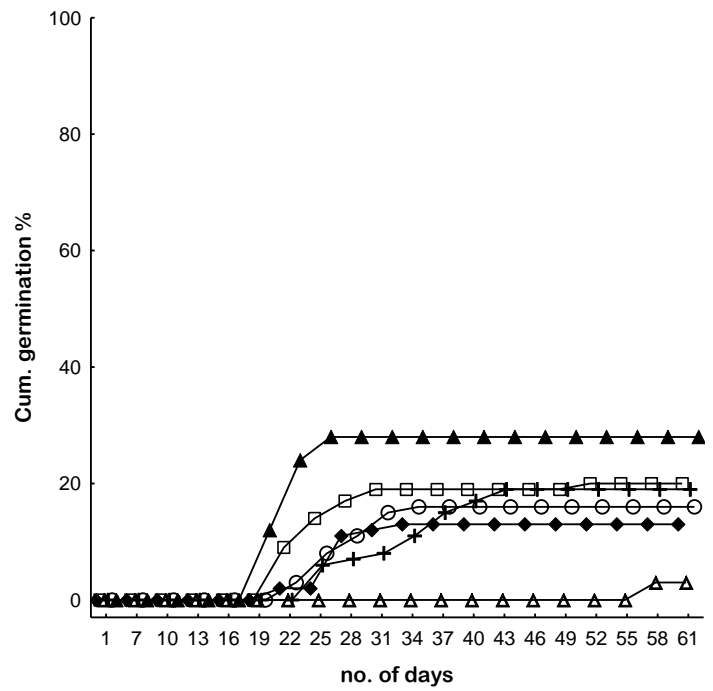
g) *Metalasia densa*



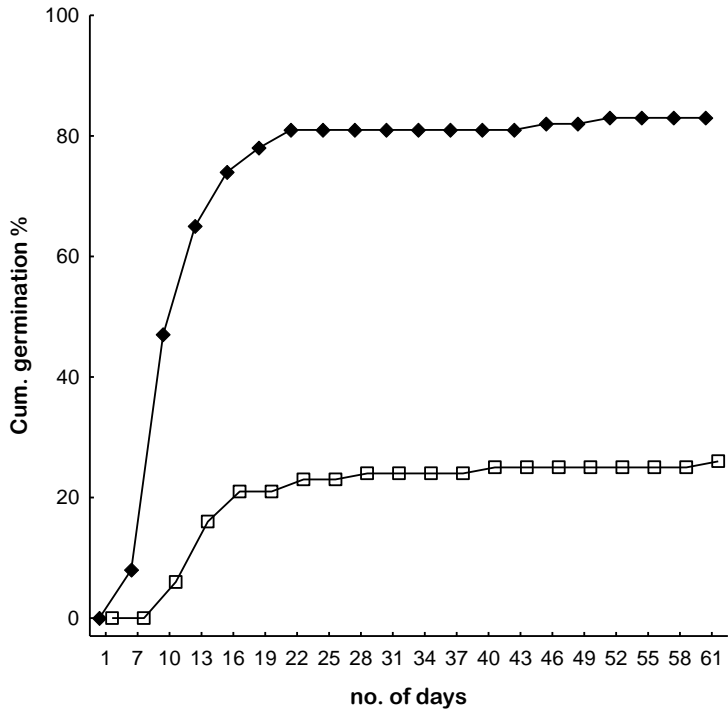
h) *Passerina corymbosa*



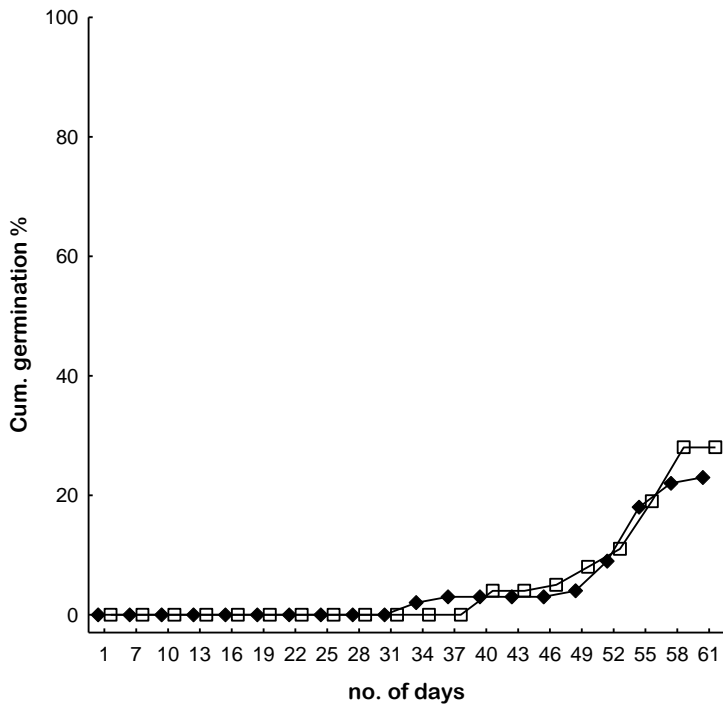
i) *Phyllica cephalantha*



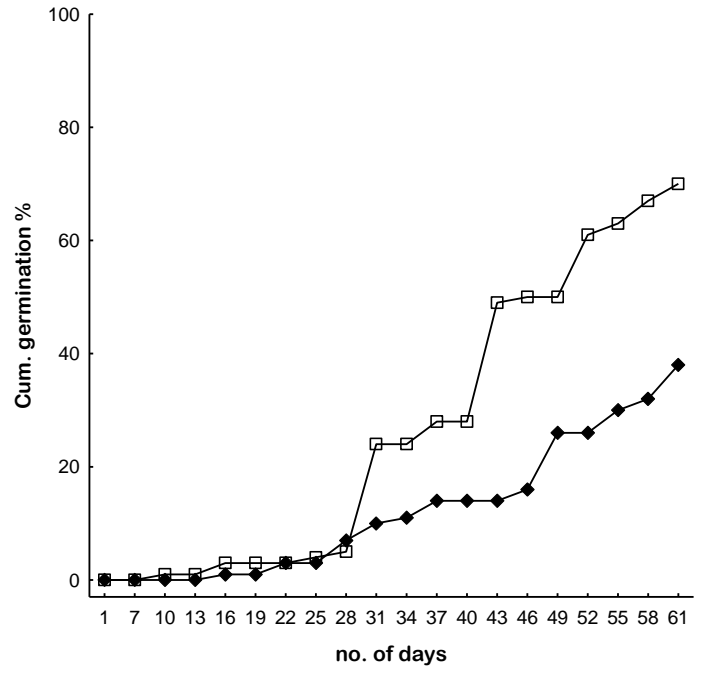
j) *Protea scolymocephala*



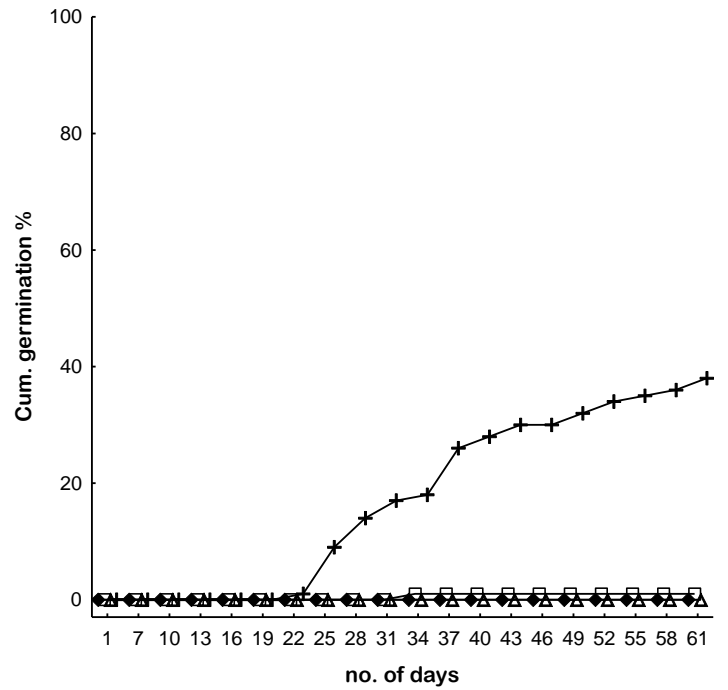
k) *Senecio elegans*



m) *Serruria fasciflora*



l) *Seriphium incanum*



n) *Thamnochortus punctatus*

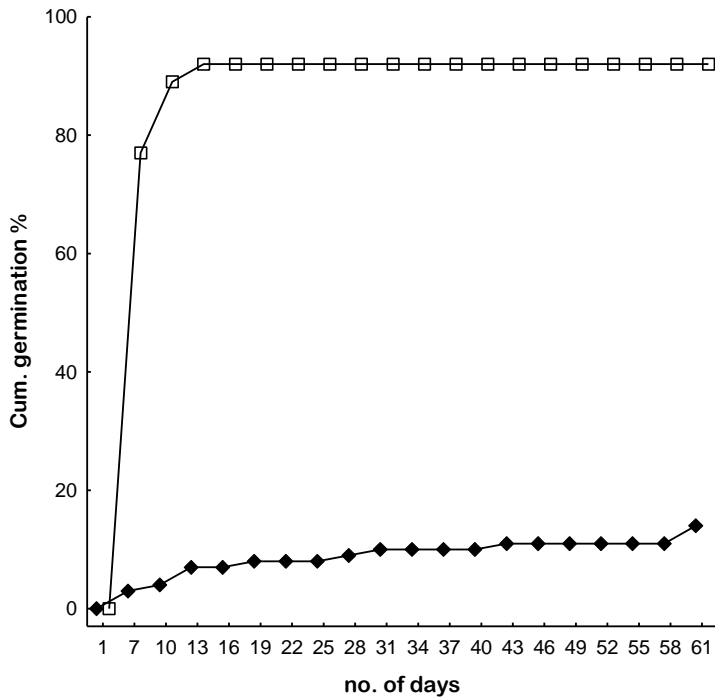
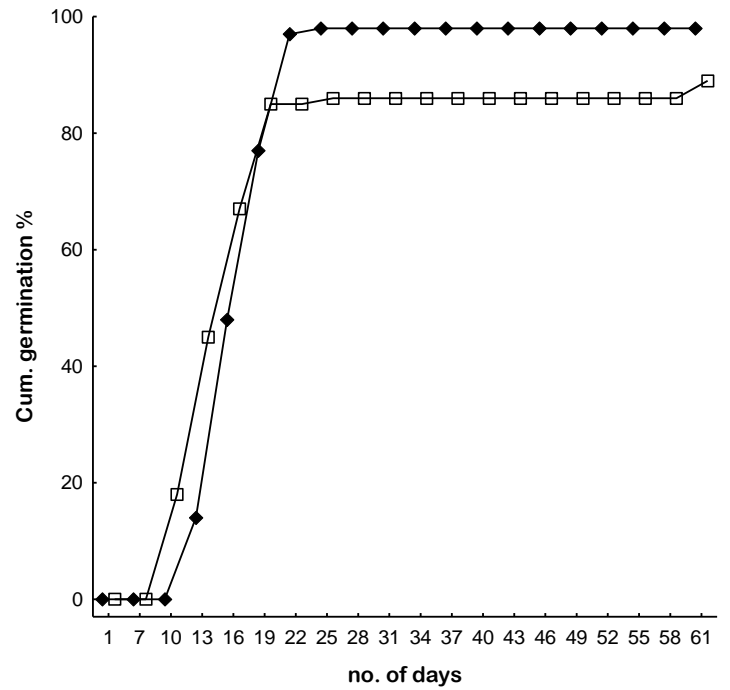
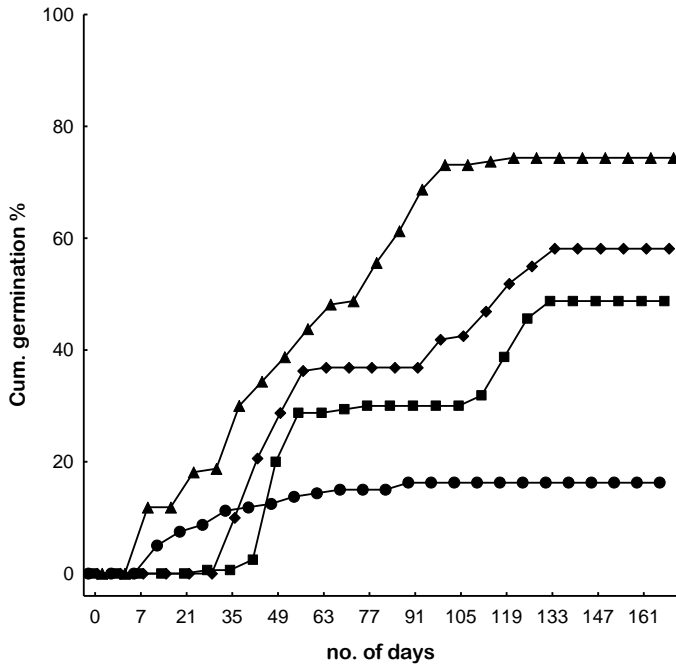
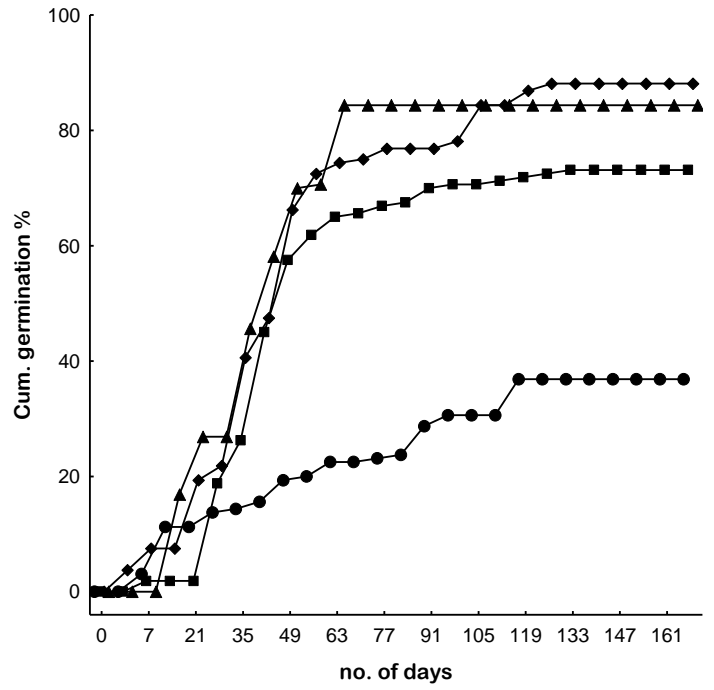
o) *Ursinia anthemoides*p) *Watsonia meriana*

Figure A.1: a-p) Cumulative curves showing percentage means of germination \pm SD, and the rate at which seeds of the target CFSF species respond to different pre-germination treatments. The x-axis is not evenly spaced at day 1 and 7, due to the fact that after experimental set-up, the first sampling was from the 7th day then continued sampling was after every 3rd day till the end of 8 weeks. The key symbols representing the treatments are shown as follows: (◆) Control, (□) Smoke water, (△) Heat, (⊕) Heat-smoke water, (⊙) Scarification, (▲) Scarification-smoke water

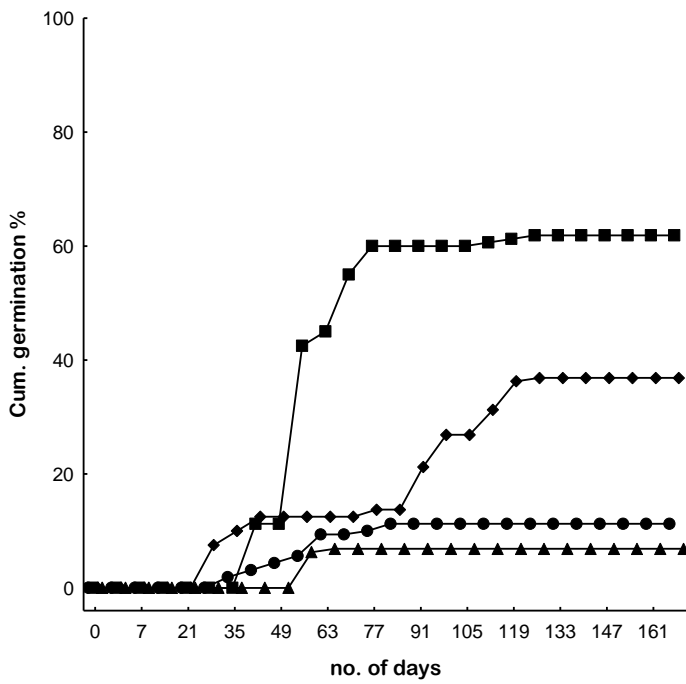
Appendix B.1



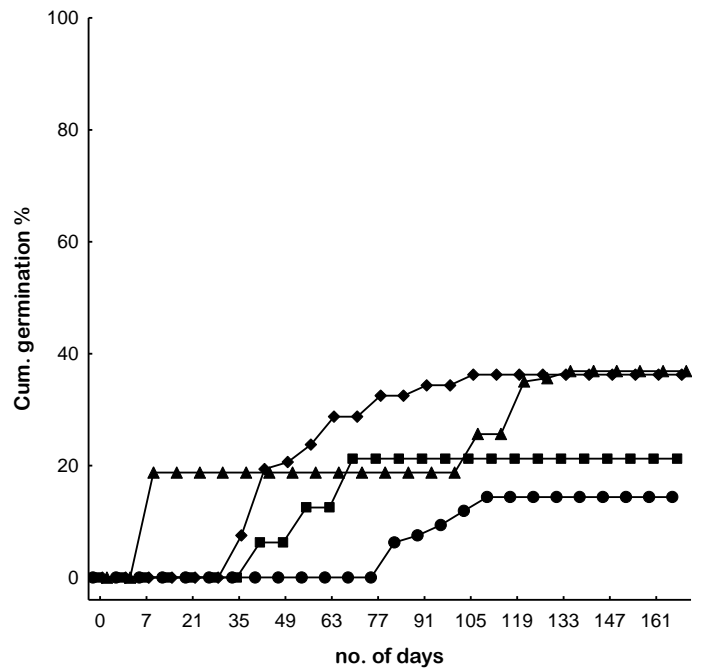
a) *Metalasia densa*



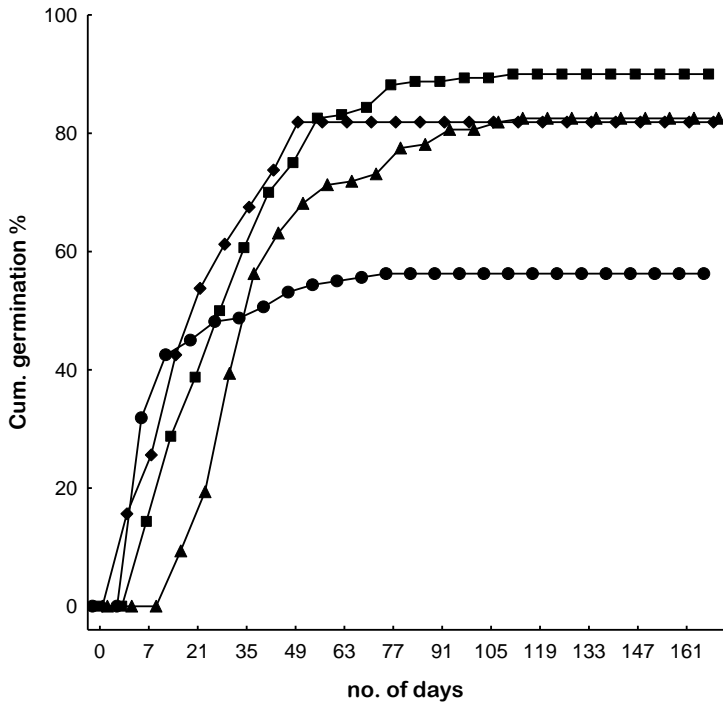
b) *Watsonia meriana*



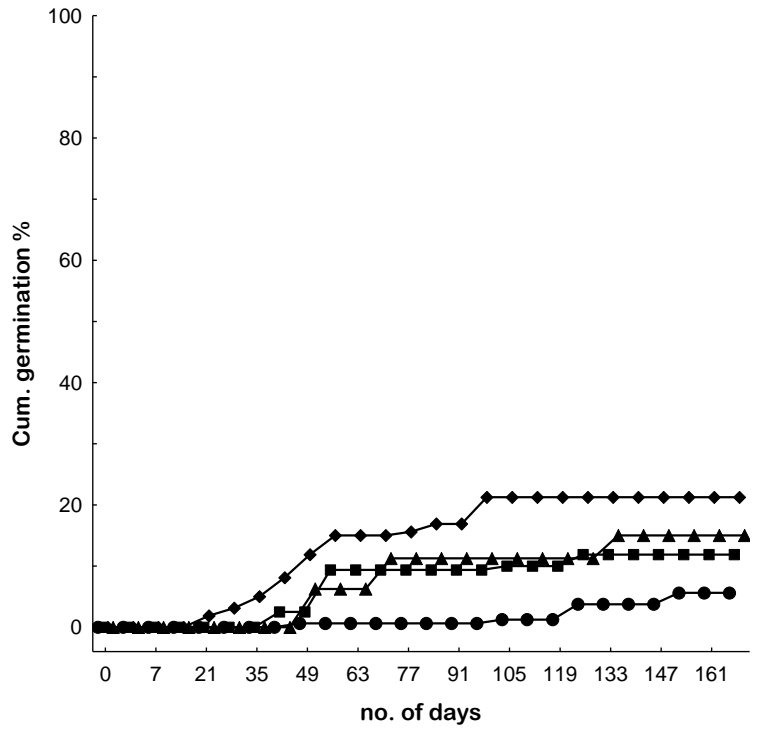
c) *Erica plumosa*



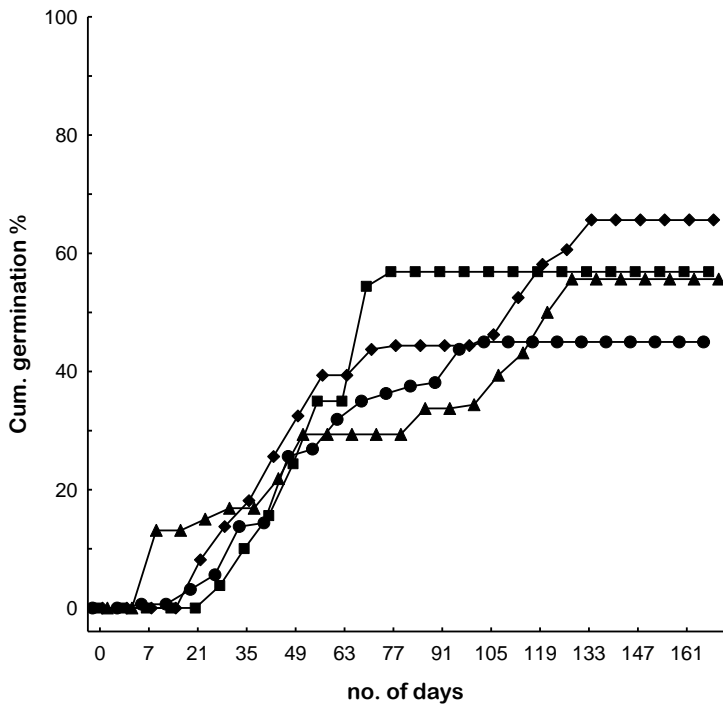
d) *Serruria fasciflora*



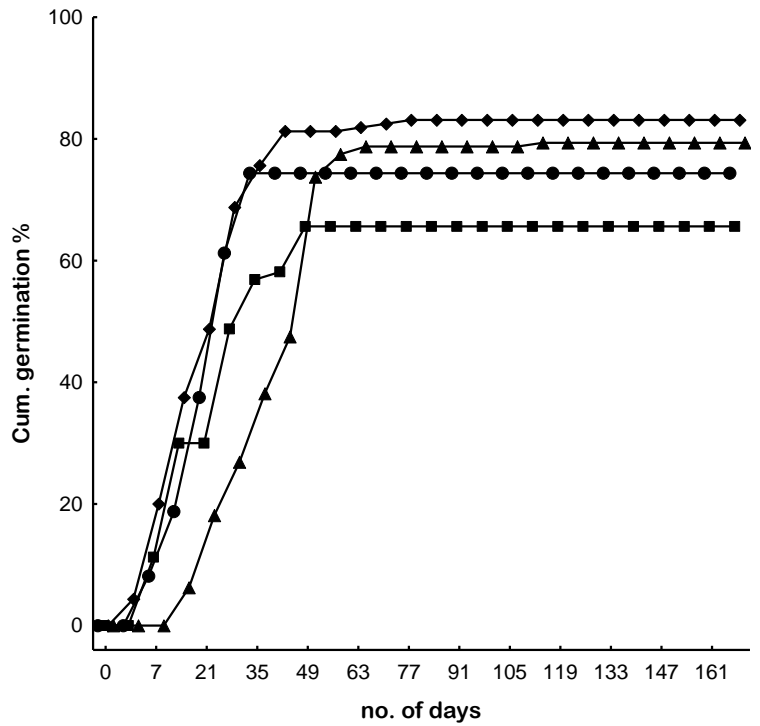
e) *Chrysocoma ciliata*



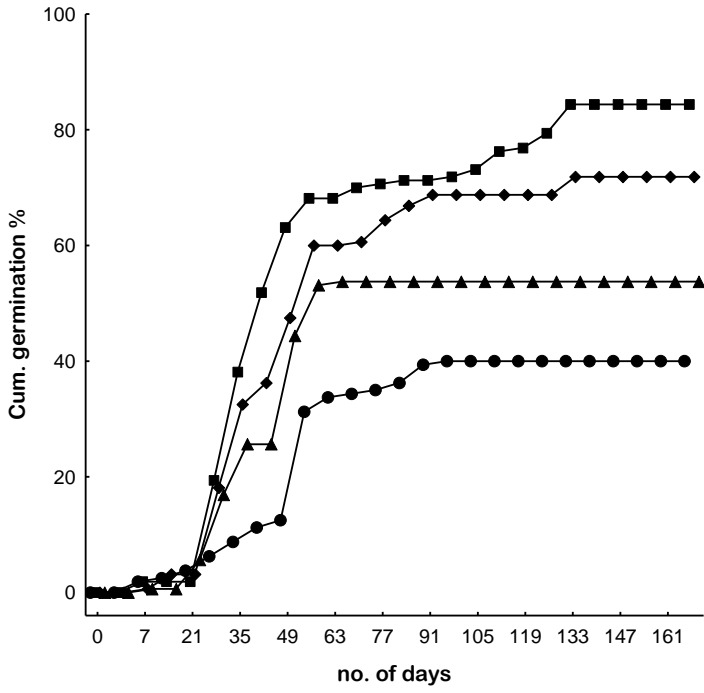
f) *Diosma oppositifolia*



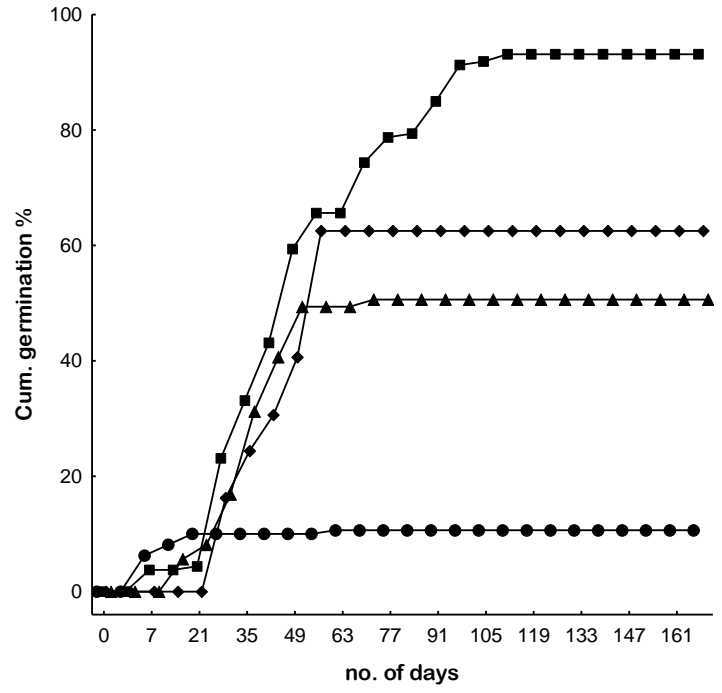
g) *Protea scolymocephala*



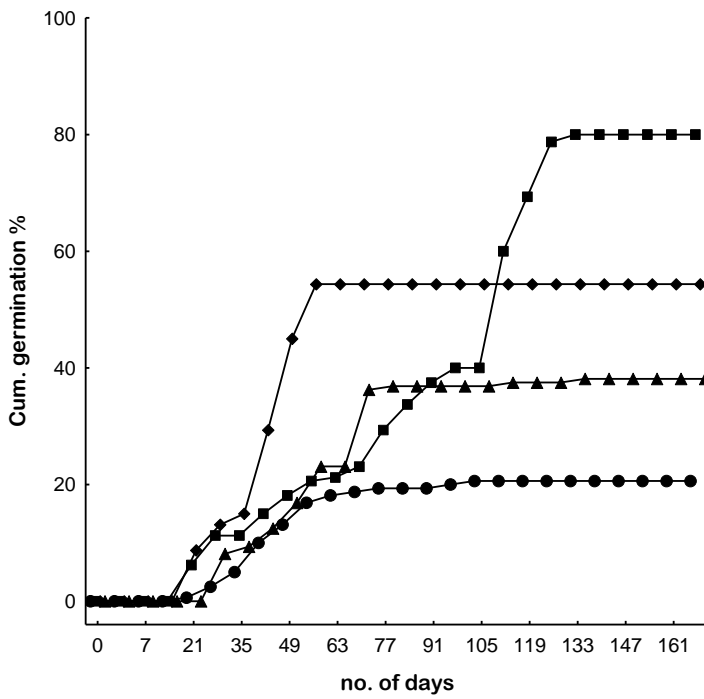
h) *Senecio elegans*



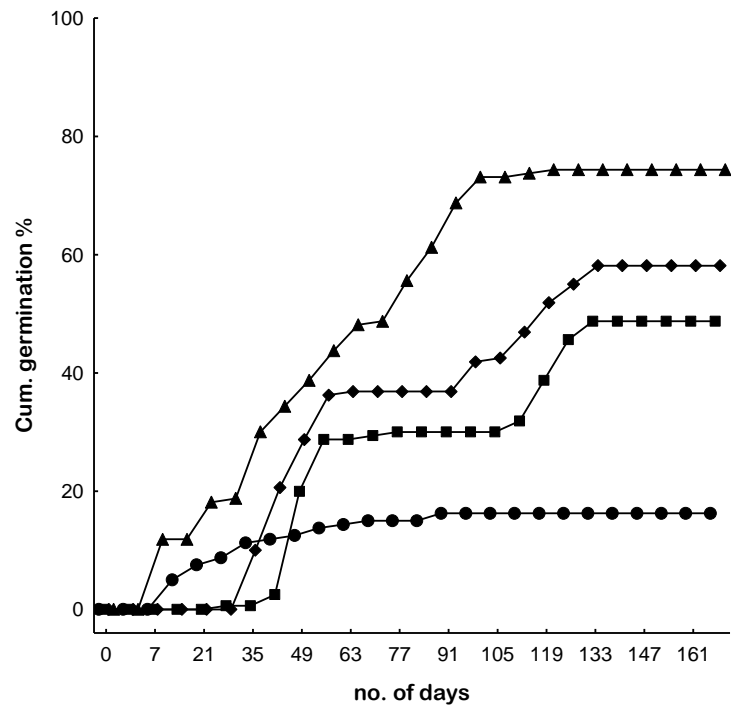
i) *Erica mammosa*



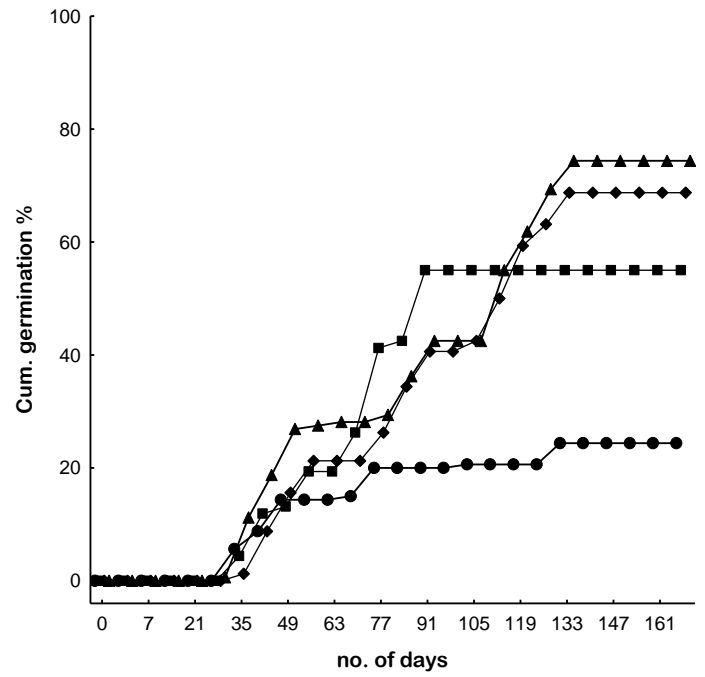
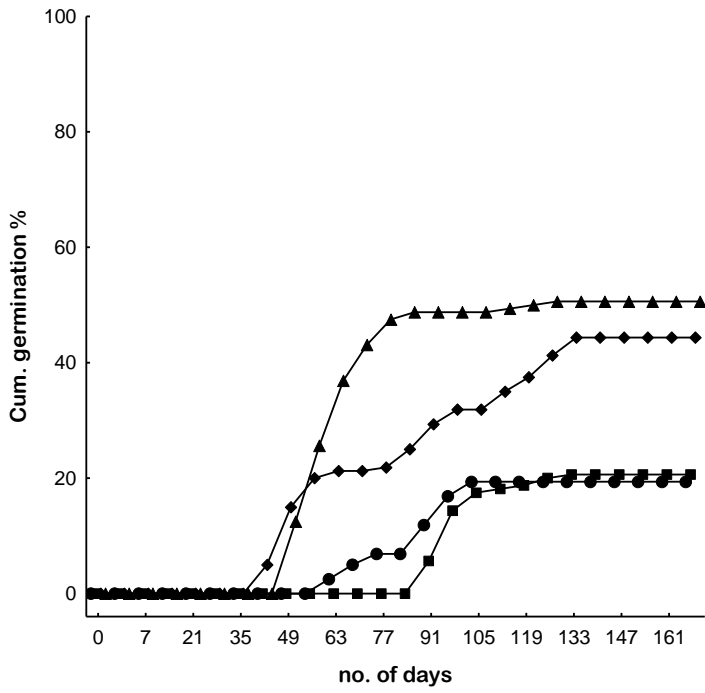
j) *Protea repens*



k) *Agathosma imbricata*

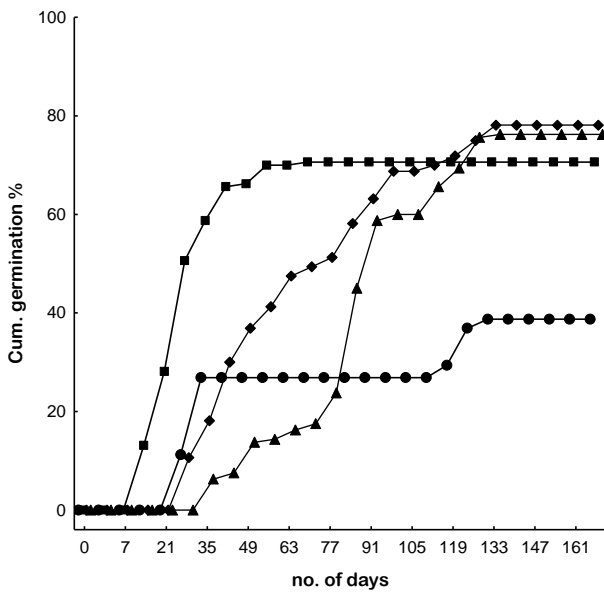


l) *Leucadendron salignum*

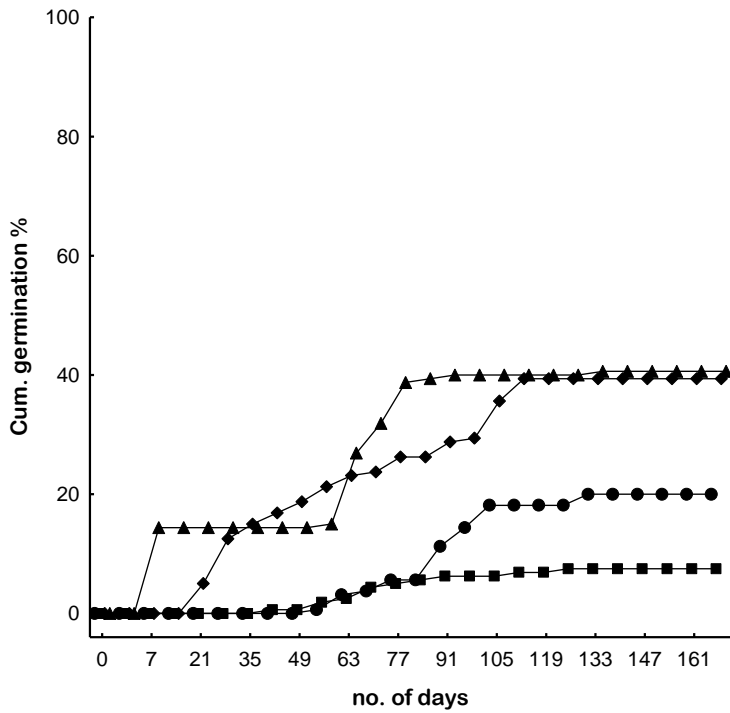


m) *Phylica cephalantha*

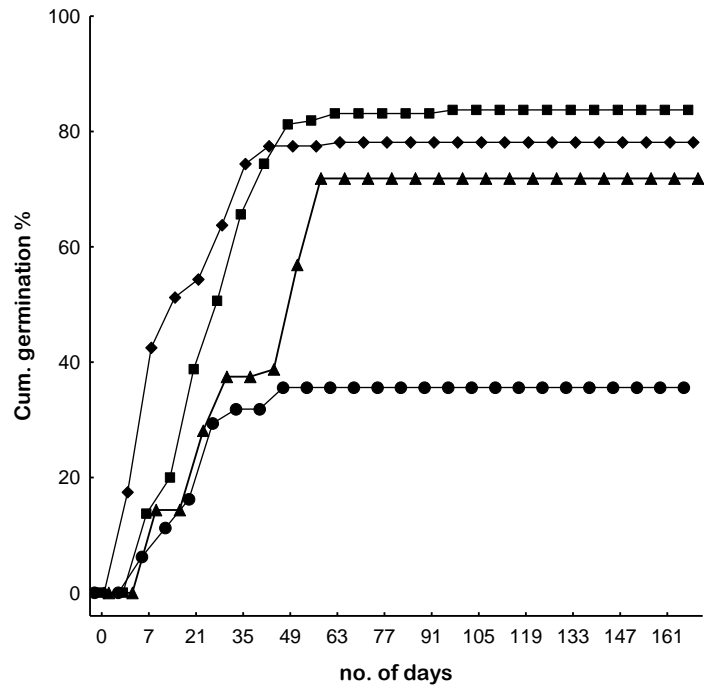
n) *Wachendorfia multiflora*



o) *Passerina corymbosa*



p) *Thamnochortus punctatus*



q) *Ursinia anthemoides*

Figure B.1. a-q): Cumulative percentage germination curves depicting germination rates of the target Cape Flats Sand Fynbos (CFSF) species about application of different plant-derived smoke pre-germination treatments. The x-axis is not evenly spaced (but the actual sampling had 6 days differences in between). The key symbols representing the treatments are shown as follows: (●) Control, (■) smoke-treated seeds, (◆), combination of smoke-treated seeds and soil, (▲) smoke-treated soil.