**Faculty of Science and Engineering**

**School of Molecular and Life Sciences**

# SEED ENHANCEMENT RESEARCH FOR IMPROVING ECOLOGICAL RESTORATION

**Simone Pedrini**

**This thesis is presented for the Degree of**

**Doctor of Philosophy**

**of**

**Curtin University**

**December 2018**

# Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgement has been made.

This thesis contains no material that has been accepted for the award of any other degree or diploma in any university.

Signature: 

Date: 25/12/2018

# Abstract

The goal of this PhD is to test seed coating technologies on native plant species to improve the efficiency of seed use in ecological restoration.

Seed coating is used to modify seed physical properties for improved handling, and deliver beneficial compounds to protect seeds from pathogens/predators, enhance germination, and promote seedling growth. Seed coating technology has been developed for the seeds of crop and horticultural species, but most of the know-how is owned by private companies and kept trade secret, making its application to native seeds particularly challenging. The lack of publicly available information on seed coating required for the re-development of seed coating methodologies. This resulted in the publication of the first openly-accessible protocol development tool (PDT) for coating seeds, a step-by-step guide for customising coating recipes and processes. The PDT, initially aimed at native seed users for testing seed coating formulations to native species, could also be employed by farmers and small seed producers that don't have access to proprietary commercial seed coating treatment.

The method described in the PDT was used to evaluate the effect of coating materials on seed germination on a test species (tomato). In the literature, seed coating was commonly reported to reduce seed germination, but the causes were not clear. Seed germination experiments, performed in controlled laboratory environment, revealed a correlation between increased mechanical integrity of the coating and reduction in germination speed. The optimal combination of materials that would maximise coat integrity with the least delay on germination was then determined.

Seed coating was tested on native grass species commonly used for pasture and ecological restoration in temperate Australia: *Austrostipa scabra, Chloris truncata, Microlaena stipoides* and *Rytidosperma geniculum*. Direct application of the coating was unfeasible due to the complex morphological features of the grass florets. Seed processing techniques for the reduction of the floret (mechanical cleaning, flash flaming and sulphuric acid digestion) were therefore tested and optimised. Sulphuric acid processing proved to be the most cost-effective method and provided the best germination outcomes. The processing of *A. scabra, M. stipoides* and *R. geniculatum* seeds allowed for the successful application of seed coating. Salicylic acid (SA), a compound known to induce stress resistance in plants, was delivered to the seed by

imbibition and coating, and its effects were tested in laboratory conditions and field trials. SA treatments were not detrimental to germination and emergence. In fact, SA treatment improved plant survival and growth in the field after the dry, summer months.

Seeds are instrumental for the reintroduction of plant species in terrestrial restoration. However, the successful use of native seed in restoration is usually limited by ecological and logistical barriers to seed germination and establishment. The results presented in this thesis highlighted the benefit of employing seed processing and coating technologies to native grass seeds. The methodologies here developed can be applied to a broader range of species and tested with a wide array of promoters to overcome the constraints that limit native seed use efficiency in ecological restoration.

# Acknowledgements

This PhD would not have been possible without the support from the Department of Environment and Agriculture that has provided a Curtin University International Postgraduate Research Scholarship. Further support was provided by the Australian Government through the Australian Research Council Industrial Transformation Training Centre for Mine Site Restoration (Project Number ICI 15000041).

Thanks to my supervisors Prof Kingsley Dixon, Dr Adam Cross, Dr Jason Stevens, Dr David Merritt for their, guidance, patience, and support through this PhD program. Special thanks to Wolfgang Lewandrowski for the invaluable assistance with the statistical analysis.

Thanks to HM Clause Vegetables, Ms Marinuccia Sartori, and Ian Chievers of Native Seed Pty Ltd, Melbourne, Australia for generously donating the seeds used throughout this thesis.

Thanks to Kings Park and Botanic Garden for giving me access to the research facilities necessary to perform seed treatment and laboratory-based experiments, and thanks to Cypress Farm for giving me access to the field site, and assistance in running the field experiments.

Thanks to the CMSR team, especially the “three moms” of the project, Renee Young, Vanessa MacDonald and Haylee D’Agui, and all of the CMSR students.

Thanks to Olga Kildisheva, Marcello De Vitis, Nancy Shaw, Rob Fiegener, Stephanie Frischie, and Kingsley Dixon for their genuine and contagious passion that had fuelled the rise and growth of the International Network for Seed Based Restoration.

Thanks to Costantino Bonomi of the NASSTEC project for supporting my travels to Europe for attending workshops, and conferences and for keeping me connected with the European native seed research network, and thanks to all of the NASSTEC students for treating me as one of them.

Thanks to Ann Kareen, Giles and Fiona, Candido and all the friends of the soon to be European Native Seed Producers Association, for the enthusiasm, commitment and energy in supporting this idea.

# Table of Content

Declaration ii

Abstract iii

Acknowledgements v

Table of Content vi

List of figures xiii

List of tables xv

List of Abbreviations xvi

General introduction 1

The need for ecological restoration 2

Native seeds for terrestrial landscape restoration 3

Seed technologies for native seeds 4

Thesis structure 5

What is seed coating? 5

How is seed coating done? 6

How does seed coating work? 7

How to make native grass seed coat-able? 7

Improving native grass seed performance with seed coating 8

Significance 8

Bibliography 9

1. Seed coating: science or marketing spin? 14

1.1. Abstract 14

1.2. What is seed coating and why is it done? 14

1.3. The case for expanding seed coating technologies to wild species 15

1.4. Equipment, materials and biological effects 16

1.4.1. Protectants 20

1.4.2. Nutrients 20

1.4.3. Symbionts 21

1.4.4. Soil adjuvants 21

1.4.5. Phytoactive promoters 24

1.4.6. Tracers and colours 24

1.5. The market for coated seed 25

1.6. Marketing benefits 27

1.7. Concluding remarks and future opportunities 28

1.8. Glossary 29

1.9. Resources 30

1.10. Bibliography 30

2. Protocol Development Tool (PDT) for seed encrusting and pelleting 38

2.1. Abstract 38

2.2. Introduction 38

2.3. Method description 40

2.3.1. General Information recording phase (GI) 41

2.3.2. Before coating phase (BC) 41

2.3.3. Coating phase (CP) 42

2.3.4. Wet phase (WP) ..... 48
2.3.5. Dry phase (DP) ..... 48
2.3.6. Quality tests (QT) ..... 48
2.3.7. Summary table ..... 49
2.4. Implications for practice ..... 50
2.5. Bibliography ..... 51
3. Seed pelleting: trade-off between germination and mechanical integrity ..... 53
3.1. Introduction ..... 53
3.1.1. Trade protection of protocols and materials ..... 53
3.1.2. Seed pelleting effect on germination ..... 54
3.2. Material and methods ..... 55
3.2.1. Species ..... 55
3.2.2. Seed pelleting ..... 56
3.2.3. Physical proprieties of pelleted seeds ..... 57
3.2.4. Germination experiments ..... 58
3.2.5. Data analysis ..... 59
3.3. Results and discussion ..... 61
3.3.1. Seed pelleting process effect on germination ..... 61
3.3.2. Physical proprieties of pelleted seeds ..... 61
3.3.3. Seed pelleting material effect on germination ..... 65
3.3.4. Trade-off between germination speed and mechanical stress resistance 69

3.4. Conclusion ..... 71

3.5. Bibliography ..... 71

4. Optimizing seed processing techniques to improve germination and sowability of native grasses for ecological restoration ..... 76

4.1. Abstract ..... 76

4.2. Introduction ..... 77

4.3. Methods ..... 79

4.3.1. Species selection and characterisation ..... 79

4.3.2. Sowability and seed delivery consistancy test ..... 80

4.3.3. Mechanical cleaning ..... 81

4.3.4. Flaming ..... 82

4.3.5. Acid digestion ..... 82

4.3.6. Germination ..... 83

4.3.7. Data analysis ..... 84

4.4. Results ..... 84

4.4.1. Seed characteristics ..... 84

4.4.2. Sowability and seed delivery consistancy test ..... 84

4.4.3. Seed processing treatment ..... 85

4.4.4. Germination tests ..... 86

4.5. Discussion ..... 89

4.5.1. Germination response and improved handling following husk removal 89

4.5.2. Seed processing methods: germination and logistics ..... 92

4.5.3. Potential for seed technology development and scaling up for restoration 94

4.5.4. Next steps ..... 94

4.6. Bibliography ..... 95

5. Seed encrusting with salicylic acid: a novel approach to improve surival of native grasses ..... 100

5.1. Introduction ..... 100

5.2. Material and methods ..... 102

5.2.1. Seed selection and cleaning ..... 102

5.2.2. Seed treatments ..... 103

5.2.3. Laboratory test ..... 104

5.2.4. Field trials ..... 104

5.2.5. Statistical analysis ..... 106

5.3. Results ..... 107

5.3.1. Encrusting and imbibition treatment ..... 108

5.3.2. Salicylic acid effects on germination with low water availability and field emergence ..... 109

5.3.3. Survival and plant growth in field site conditions ..... 111

5.4. Discussion ..... 113

5.4.1. Seed treatment effects on germination and emergence ..... 113

5.4.2. Salicylic acid effect on seed germination and emergence ..... 113

5.4.3. Survival and growth ..... 114

5.5. References ..... 117

General discussion ..... 123
Transparency is needed in the seed coating industry ..... 123
Impact of seed coating constituents on germinability of seeds ..... 124
Application of seed enhancement to native species ..... 125
Further research ..... 126
Implication for ecological restoration ..... 127
Conclusion ..... 128
Bibliography ..... 129
Appendices ..... 133
S.1. Supplemental Information: Chapter 1 ..... 133
S.1.1. Seed coating/pelleting techniques ..... 134
S.1.2. Film coating polymers and binding material ..... 135
S.1.3. Filler material used for encrusting and pelleting ..... 136
S.1.4. Effects of seed coating with protectants ..... 137
S.1.5. Effects of seed coating with nutrients ..... 138
S.1.6. Effects of seed coating with symbiotic organism. ..... 139
S.1.7. Seed coating products and services analysed divided by companies ..... 140
S.1.8. List of publications used in the review process ..... 141
S.2. Activities related to this thesis ..... 153
S.2.1. Publications ..... 153
S.2.2. Conference presentations ..... 157
S.2.3. Interactive Recovery Wheel App ..... 158

S.2.4. International Network for Seed Based Restoration ..... 164

S.3. Paper co-authorship statement ..... 165

S.4. Copyright permission statement ..... 168

# List of figures

Figure 0.1: General questions and grass species testes. The general questions are sequentially presented in the chapter of the thesis. ..... 6

Figure 1.1: Seed coating ingredients, equipment and coat type.. ..... 18

Figure 1.2: Differences among seed industry and academia in terms of species evaluated, seed coating ingredients employed and outcomes or claims effects of the coating treatment. ..... 23

Figure 2.1: Pro forma to be filled by the operator during encrusting or pelleting..... 43

Figure 2.2: Laboratory setting, equipment and tools required for the development of seed pelleting or encrusting protocols. ..... 44

Figure 2.3: Diagram illustrating the main components of a rotary coater. ..... 45

Figure 2.4: flow chart of seed pelleting and encrusting process. ..... 45

Figure 2.5: Images of (top row) tomato seeds and (bottom row) Microlaena stipoides seeds at the end of the different stages of the coating process. ..... 47

Figure 3.1: Correlation between pellet mechanical resistance to stress (N) and concentration of the binder hydroxyl ethylcellulose (HEC). ..... 63

Figure 3.2: Weight increase of pelleted seed imbibed at two different water potentials ..... 64

Figure 3.3: Seed germination at two different water potentials..... 67

Figure 3.4: Correlations between germination performance of pelleted seed and mechanical stress resistance.. ..... 68

Figure 3.5: Trade-off analysis graph for the optimal binder concentration percentage that maximises mechanical resistance whilst minimising delay in germination. ..... 70

Figure 4.1: Floret and caryopsis of the test species ..... 81

Figure 4.2: Cumulative germination curve of floret and caryopsis. Results were estimated using a three parameter log-logistic curve. ..... 88

Figure 4.3: Final germination percentage and germination speed as days required to reach 50% of the final germination (T50) ..... 90

Figure 4.4: Cumulative germination percentage curve of the three different seed processing methods, MC = manual cleaning, AC = acid digestion and FL = flaming..... 91

Figure 5.1: Seeds of the three grass species tested. ..... 104

Figure 5.2: Climate condition at the field site. ..... 106

Figure 5.3: Cumulative germination/emergence percentage curve of the three different seed treatment tested. ..... 108

Figure 5.4: Final germination and emergence proportion of untreated used (Ctrl), seed treated without Salicylic Acid (No) and seed treated with Salicylic acid (SA) ..... 110

Figure 5.5: Survival and plant growth comparison 40 weeks after sowing ..... 112

Figure 5.6: Cumulative survival proportion of sown seeds in the line experiment ..... 115

Figure S.1: Seed coating/pelleting technique ..... 134

Figure S.2: Film coating polymers and binding material. ..... 135

Figure S.3: Filler material used for encrusting and pelleting. ..... 136

Figure S.4: Effects of seed coating with protectants ..... 137

Figure S.5: Effects of seed coating with nutrients. ..... 138

Figure S.6: Effects of seed coating with symbiotic organism. ..... 139

Figure S.7: Seed coating products and services analysed divided by companies ..... 140

# List of tables

Table 1.1: Effects of Phytoactive compounds ..... 25

Table 2.1: Description of the six phases of the Protocol Development Tool and list of the equipment required at each phase ..... 42

Table 3.1: Seed pelleting process specification for the ten pelleting treatments.. ..... 62

Table 4.1: Exposure time to different treatment per species ..... 83

Table 4.2: Characterization of floret and cleaned caryopsis in four Australian grass species. ..... 85

Table 4.3: Direct seeding results (Earthway Precision Garden Seeding machine) comparing floret and cleaned seed. ..... 86

# List of Abbreviations

AC: acid digestion

BC: before coating phase

BND: binder

CP: coating phase

CTRL: control

DE: diatomaceous earth

DP: dry phase

ENCR: encrusting

FL: flash flaming

GI: general information recording phase

IMB: imbibition

HEC: hydroxyethyl cellulose

MC: manual cleaning

QT: quality test

PDT: protocol development tool

PEG: polyethylene glycol

PWD: powder

SA: salicylic acid

TL: talc

WP: wet phase

# General introduction

Healthy ecosystems provide environmental values and services that benefit humanity and, in many cases, are essential to those people still connected to natural ecosystem services (Palmer and Filoso 2009). However, humans have developed technologies and capacities that have disconnected them from natural systems. The ability to control fire for cooking, warmth and keeping predators away (Lewis 1960), along with the domestication of plant and animal species for farming, building huts and houses, and organising complex social structures has allowed humanity to grow in number and expand to environments well beyond their pre-technological capacity (Harari 2014). As a result, through post-agrarian history, humans have been actively and increasingly altering and modifying ecosystems both inadvertently and purposely. Human capability to alter ecosystems is usually dependant on their level of technological advancement (Western 2001).

The advent of scientific enquiry in the 17th century has provided the perfect conditions to fuel an unprecedented level of scientific and technological innovation. The industrial revolution enabled human populations to move into artificially designed and controlled urban settings. Innovations in health science have decreased mortality, whilst advancement in agriculture have increased food availability (Van Bavel 2013). In combination, these factors triggered rapid growth in human population from 1.05 billion in 1820 to 7.33 in 2016, and population growth is expected to plateau by the end of the century, at 11.2 billion (https://ourworldindata.org/world-population-growth).

The explosion of the human population, along with the rising need for land and resources to support consumer-based societies, is putting natural ecosystems under increasing pressure, and at times threatening their existence. Yet healthy ecosystems are those that provide the goods and services, such as climate regulation, water regulation and supply, erosion control, soil formation, food and raw material production that sustain humankind survival and wellbeing. According to Costanza et al. (1997) the value that the biosphere provides to humanity, in monetary term, is estimated at an average of 33 trillion $/year, which was almost double the global GDP at the time the study was conducted. Unfortunately, such value is usually not accounted for in the majority of projects and programs that are directly or indirectly responsible

for ecosystem degradation via modification of their composition, alteration of their function and, in some case, their complete destruction.

## The need for ecological restoration

Nearly two-thirds of the world ecosystems are considered degraded, damaged or impacted by humans to varying degrees. Often these systems are subjected to mismanagement, and a lack of restorative effort and investment to compensate for destructive activities means that the degraded states persist or are further exacerbated (Nellemann and Corcoran 2010).

The most effective way to preserve the natural capital of healthy ecosystems is through conservation measures. However, just 15% of terrestrial, 10% of coastal and marine and 4% of ocean ecosystems are under protection globally (UNEP-WCMC and IUCN 2016), leaving most of the planet potentially vulnerable to human-induced degradation. Loss of biodiversity and ecosystem complexity, in degraded landscapes, often results in ecosystems with reduced functionality, ability to self-sustain (ecosystem stability), withstand stresses (resistance), and recover from disturbances (resilience) (Society for Ecological Restoration International Science & Policy Working Group 2004). Once degradation has occurred, ecosystems could be recovered or reinstated through the practice of ecological restoration (Jordan and Lubick 2001; Clewell and Aronson 2007). In the SER international primer on ecological restoration (Society for Ecological Restoration International Science & Policy Working Group 2004) ecological restoration was defined as "*the process of assisting the recovery of an ecosystem that has been degraded, damaged or destroyed*". However, this definition did not have clear, practical meaning and, until recently, the term *ecological restoration* was used interchangeably with revegetation, rehabilitation, and remediation (Cross et al. 2018). With the publication of the Australian National Standards for the Practice of Ecological Restoration (McDonald et al. 2016) and its international version (Mcdonald et al. 2016), ecological restoration was better defined, and the concept of an appropriate, local indigenous reference ecosystem was introduced as the outcome to which any restoration project should aspire to (Gann et al. 2018). A tool for the evaluation of how the restoration of a specific degraded ecosystem is performing, compared to the reference ecosystem, was developed (Appendix S.2.3), allowing for a standard and comparable methodology to practically and effectively evaluate

restoration performance, and assess restoration trajectory (McDonald and Dixon 2018).

## Native seeds for terrestrial landscape restoration

Ecological restoration is underpinned by the successful reinstatement of appropriate vegetation (Barr et al. 2017) that reflects a native or analogue reference ecosystem (Gann et al. 2016). Whilst species composition, density and spatial distribution over the landscape could be inferred from the reference ecosystem (McDonald et al. 2016), the positive outcome of a restoration project is usually achieved through the use of appropriate propagation material, either as green stock (seedlings/plants) or seeds (Vander Mijnsbrugge et al. 2010).

Green stock is a popular option because of good survival rates and immediate impact (Commander et al. 2013). On the downside, the process of cultivation, transport and planting adds expense and might not be feasible on broad scale projects, particularly with initiatives such as the Bonn Challenge that propose 150 million hectares of restored landscapes (Forest and Landscape Restoration 2013). Seeds represent the more cost-effective way for reintroducing plant species and are the primary tool for terrestrial restoration (Merritt and Dixon 2011).

However, the successful use of native seed in restoration is usually limited by seed availability and quantity, logistical issues (collection, processing, storage, and sowing), and dormancy/physiological/ecological barriers to seed germination and establishment.

In many countries, the majority of seeds available for restoration are directly harvested from the wild. In the long-term, this approach is potentially unsustainable given the global scale of restoration and negative impacts on the ecological integrity of remnant wild areas (Meissen et al. 2015, 2017). In addition, wild collection is unlikely to be sufficient to fulfil the rising demand for native seeds (Broadhurst et al. 2015; Nevill et al. 2018). As a result, the cost of native seed is often prohibitive (Merritt and Dixon 2011), and seeds are rarely available in the quantity and diversity required (Wijdeven and Kuzee 2000; Broadhurst et al. 2008). In countries like the United States, where native seeds are produced in large-scale farms (Gibson-Roy 2018), and seed supply is usually reliable, there are still severe limitations on how native seeds perform in degraded landscapes. Recent studies have suggested that the expected successful

establishment of plants grown from direct seeding is usually below 10% (Turner et al. 2006; James et al. 2011; Ceccon et al. 2016). Numerous variables such as seed quality, seed dormancy, site condition, biotic and abiotic stresses and competition can affect seed-based restoration outcomes (Madsen et al. 2016). Moreover, native seeds are inherently highly variable in size and shape and therefore pose logistical challenges to seed producers and restoration practitioners (Loch 1993; Guzzomi et al. 2016). For example, seeds may have appendages, such as hairs and awns, that would make the process of admixing problematic, and precision sowing unfeasible (Alizadeh et al. 2012; Waters et al. 2017).

If ecological restoration has to be delivered at the scale required (Menz et al. 2013), there is an impelling need to improve the efficiency of native seeds use so that the estimated failure rates (>90%) of seed in restoration (Merritt and Dixon, 2011, Menz et al. 2013) can be reversed.

## Seed technologies for native seeds

The application of seed enhancement technologies to native species could provide a solution to some of the native seeds shortcomings in restoration scenarios. These technologies have been successfully deployed in the horticultural and agricultural fields for more than 50 years, and have resulted in substantial improvements in seeding success and seedling establishment (Halmer 2008).

The two main approaches in seed enhancements are seed priming and seed coating. Seed priming consists of the controlled hydration of seeds, which triggers pre-germinative metabolic activities, improving germination speed and synchronicity (Paparella et al. 2015). Seed coating relies on the application of external material to the seed to deliver potentially beneficial compounds (protectants, germination promoters, growth enhancers) and modify seed shape and size to improve handling (Scott 1989; Taylor et al. 2004). This thesis will focus mostly on seed coating.

The aim of this thesis was to research seed coating technologies, materials, and adjuvants for addressing logistical and ecological constraints that limit the efficient use of native seeds. Four native Australian grass species (*Austrostipa scabra* (Lindl.) S.W.L.Jacobs & J.Everett , *Chloris truncata* R.Br., *Microlaena stipoides* (Labill.) R.Br. var. Griffin and *Rytidosperma geniculum* (J.M.Black) Connor & Edgar var. Oxley) (Figure 0.1) were chosen as the study species due to their widespread use in ecological restoration

and revegetation in temperate Australia (Loch et al. 1996). The selected grasses are also representative of an array of complex external morphological features (floret) that confound current seeding methods. Thus, the four study taxa are ideal for the testing of the efficacy of seed enhancement technologies in addressing potential logistical impediments.

However, before seed coating technologies can be applied to native grass seeds, there is the need to identify what technologies are currently available and test those that are best suited for native seeds. This thesis aims to address this by reviewing the current knowledge on seed coating, developing appropriate protocols for the application of seed coating onto native seeds and testing the efficacy of seed coating treatment in improving native grass seed establishment.

## Thesis structure

This thesis is composed of five chapters: a literature review, a methodology chapter and three experimental chapters (Figure 0.1). The structure represents a logical progression from an analysis of seed enhancement potentials for native seed through to the development of materials and methods for building seed coats.

### *What is seed coating?*

The first step is to investigate how the crop and vegetable seed science and industry have addressed seed performance issues with seed coating, and what could the native seed sector learn and adopt from agriculture. This chapter contains a literature review, published in February 2017 in the Journal "Trends in Plant Science"[1], that provides general definitions and descriptions of the most commonly used material and methods. It also highlights that a handful of major agrochemical companies withhold most of the technological capabilities and know-how, and do not share them with academia and the public.

[1] Pedrini S, Merritt DJ, Stevens J, Dixon K (2017) Seed Coating: Science or Marketing Spin? Trends Plant Sci 22:106–116. doi: 10.1016/j.tplants.2016.11.002

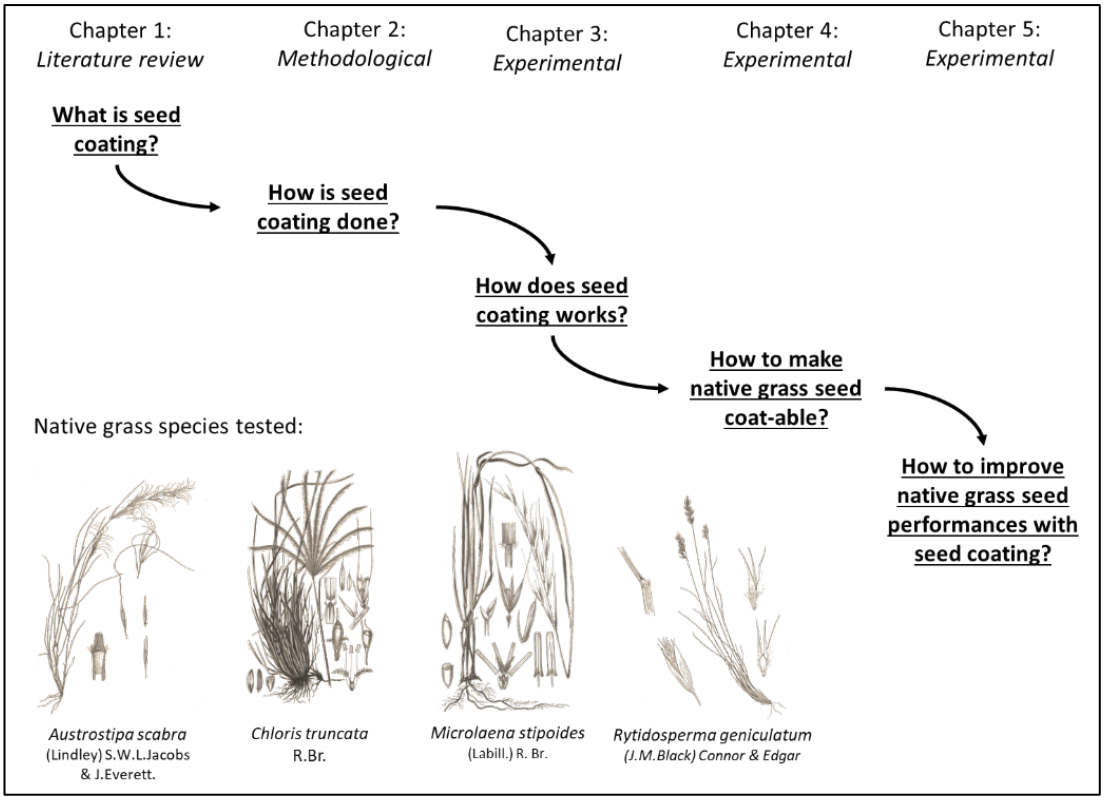



*Figure 0.1: General questions and grass species tested. The general questions are sequentially presented in the chapters of the thesis.*

## *How is seed coating done?*

The lack of publicly available seed coating protocols has made the evaluation and application of seed coating to native seed unfeasible. A protocol development tool for seed coating (pelleting and encrusting) was developed by testing different coating materials and refining coating techniques on a test-crop (tomato) and a native species (*Microlaena stipoides*). This chapter was published in July 2018 in the Journal "Seed Science and Technology"[2]. The document provides the first detailed analysis and step-by-step guide to the process of seed encrusting/pelleting, along with the list of material and equipment needed, a standardised Pro-forma to record every step of the process, and an excel spreadsheet to keep track of various trails and to allow for the development of species-specific protocols.

[2] Pedrini S, Bhalsing K, Cross AT, Dixon KW (2018) Protocol Development Tool (PDT) for seed encrusting and pelleting. Seed Sci Technol 46:393–405. doi: 10.15258/sst.2018.46.2.21

## *How does seed coating work?*

Once the methodology for encrusting and pelleting seeds was consolidated, a further problem to address was how seed pelleting affected seed germination. It has been commonly reported in the literature that coated seed (pellets) would have lower or slower germination than the untreated control. However, the mechanism underlying this phenomenon is not yet clear. I tested the hypothesis that mechanical proprieties of the coated seeds, resulting from the different combination of materials, would delay germination and identified the optimal material combination that would allow for the best mechanical properties with the least detriment on germination speed.

## *How to make native grass seed coat-able?*

Seed coating protocols and materials identified in the previous chapters were tested on seeds of Australian native grasses: *A. scabra, C. truncata, M. stipoides* and *R. geniculum*. However, the coating process was inefficient and time-consuming because of the complex morphological features of native grass seed (florets). A floret* is composed of a caryopsis that is encapsulated in an external structure (husk) that usually presents various appendices such as hairs and awns. The floret in a natural setting could provide for the dispersal and self-burial of the seed. However, when seeds are meant to be processed and sown (as in a restoration scenario) such structures significantly limit handling and deployment. Moreover, some studies suggested that the husk could limit germination (Lewandrowski et al. 2017). In this study, I have compared two previously described techniques that rely on exposure to flame (Guzzomi et al. 2016) and acid digestion (Stevens et al. 2015) to reduce floret appendages and customised the process to remove the husk completely. Generally, husk removal improved seed germination and sulphuric acid processing proved to be the most time-effective treatment while providing the best germination outcomes. These findings were published in the Journal "Plant Biology" in August 2018[3].

---

[3] Pedrini S, Lewandrowski W, Stevens JC, Dixon KW (2018) Optimizing seed processing techniques to improve germination and sowability of native grasses for ecological restoration. Plant Biol. doi: 10.1111/plb.12885

** A floret is a part of the inflorescence. The botanically correct terminology to describe the seed dispersal unit is diaspore. However, in most of the published literature, the seed dispersal unit in grasses is referred as florets, other than diaspore. The term "floret" used through the thesis is to be intended as dispersal unit of grass seed (diaspore).*

### *Improving native grass seed performance with seed coating*

Once seeds have been processed in a way that improves handling and germination, seed coating was tested on the three native grass species (*A. scabra, M. stipoides, and R. geniculata*), following the PDT described in chapter 2 and using the optimal material combination identified in chapter 3. The coat was loaded with a compound that is known to improve plant resistance to biotic and abiotic stress: salicylic acid (Senaratna et al. 2000; Stevens et al. 2006; Janda et al. 2007). Although the effect of this potent anti-stress compound has been widely investigated on crop and vegetable species, its application to native species has rarely been tested, and never delivered via seed coating. When tested in the field, plants grown from seed that had been treated with salicylic acid survived more through the drought cycles of the first summer after establishment and had higher biomass production than the untreated control.

## Significance

The findings of this research could provide the blueprint for further development and evaluation of seed coating technologies to native seeds.

The re-definition of what seed coating is, the publication of the seed coating protocol development tool and description of how pelleting materials behave, could help scientists in developing seed coating technologies capable of overcoming logistical and physiological barriers in the effective deployment of native seed. In addition, the outcomes in the thesis provide small seed companies (agricultural and native species) with seed enhancement capabilities, as they usually do not have access to the proprietary commercial seed coating treatment nor the ability to experiment and develop their own seed coating solutions.

The seed processing methods evaluated in this thesis could impact the way grass seeds, and seed with similar logistical issues, are stored and used, and seed enhancement, achieved through seed coating, could give native seed companies/restoration practitioners the capability to test and scale-up seed treatments in order to improve seeding efficiency and, ultimately, increase the success of seed based restoration projects.

# Bibliography

Alizadeh MR, Minaei S, Rahimi-Ajdadi F, et al (2012) Flow properties of awned and de-awned paddy grains through a horizontal hopper orifice. Part Sci Technol 30:. doi: 10.1080/02726351.2011.584128

Barr S, Jonas JL, Paschke MW (2017) Optimizing seed mixture diversity and seeding rates for grassland restoration. Restor Ecol. doi: 10.1111/rec.12445

Broadhurst L, Driver M, Guja L, et al (2015) Seeding the future - the issues of supply and demand in restoration in Australia. Ecol Manag Restor 16:29–32. doi: 10.1111/emr.12148

Broadhurst LM, Lowe A, Coates DJ, et al (2008) Seed supply for broadscale restoration: maximizing evolutionary potential. Evol Appl 1:587–597. doi: 10.1111/j.1752-4571.2008.00045.x

Ceccon E, González EJ, Martorell C (2016) Is Direct Seeding a Biologically Viable Strategy for Restoring Forest Ecosystems? Evidences from a Meta-analysis. L Degrad Dev 27:511–520. doi: 10.1002/ldr.2421

Clewell A, Aronson J (2007) Ecological Restoration

Commander LE, Rokich DP, Renton M, et al (2013) Optimising seed broadcasting and greenstock planting for restoration in the Australian arid zone. J Arid Environ 88:226–235. doi: 10.1016/j.jaridenv.2012.08.012

Costanza R, D'Arge R, de Groot R, et al (1997) The value of the world's ecosystem services and natural capital. Nature 387:253–260. doi: 10.1038/387253a0

Cross AT, Young R, Nevill P, et al (2018) Appropriate aspirations for effective post-mining restoration and rehabilitation: a response to Kaźmierczak et al. Environ Earth Sci 77:256. doi: 10.1007/s12665-018-7437-z

Forest and Landscape Restoration (2013) The Bonn Challange. http://www.forestlandscaperestoration.org/topic/bonn-challenge

Gann GD, McDonald T, Aronson J, et al (2018) The SER Standards: a globally relevant and inclusive tool for improving restoration practice-a reply to Higgs et al. Restor Ecol 26:426–430. doi: 10.1111/rec.12819

Gibson-Roy P (2018) Restoring grassy ecosystems - Feasible or fiction? An inquisitive Australian's experience in the USA. Ecol Manag Restor 19:11–25. doi: 10.1111/emr.12327

Guzzomi AL, Erickson TE, Ling KY, et al (2016) Flash flaming effectively removes appendages and improves the seed coating potential of grass florets. Restor Ecol 24:S98–S105. doi: 10.1111/rec.12386

Halmer P (2008) Seed Technology and Seed Enhancement. Acta Hortic 771:17–26

Harari YN (2014) Sapiens: A Brief History of Humankind. McClelland & Stewart

James JJ, Svejcar TJ, Rinella MJ (2011) Demographic processes limiting seedling recruitment in arid grassland restoration. J Appl Ecol 48:961–969. doi: 10.1111/j.1365-2664.2011.02009.x

Janda T, Horváth E, Szalai G, Páldi E (2007) Role of salicylic acid in the induction of abiotic stress tolerance. In: Salicylic Acid: A Plant Hormone. pp 91–150

Jordan WR, Lubick GM (2001) Making Nature Whole : A History of Ecological Restoration. Island Press/Center for Resource Economics, Washington, DC

Lewandrowski W, Erickson TE, Dixon KW, Stevens JC (2017) Increasing the germination envelope under water stress improves seedling emergence in two dominant grass species across different pulse rainfall events. J Appl Ecol 54:997–1007. doi: 10.1111/1365-2664.12816

Lewis R (1960) The evolution man, or, How I ate my father. New York, NY

Loch DS (1993) Tropical pasture establishment. 5. Improved handling of chaffy grass seeds: options, opportunities and value. Trop Grasslands 27:314–326

Loch DS, Johnston PW, Jensen TA, Harvey GL (1996) Harvesting, processing, and marketing Australian native grass seeds. New Zeal J Agric Res 39:591–599. doi: 10.1080/00288233.1996.9513218

Madsen MD, Davies KW, Boyd CS, et al (2016) Emerging seed enhancement technologies for overcoming barriers to restoration. Restor Ecol 24:77–84. doi: 10.1111/rec.12332

McDonald T, Dixon K (2018) National standards: Reasserting the ecological restoration framework in uncertain times. Ecol Manag Restor 0:1–11. doi: 10.1111/emr.12317

Mcdonald T, Gann GD, Jonson J, Dixon KW (2016) International standards for the practice of ecological restoration – including principles and key concepts

McDonald T, Jonson J, Dixon KW (2016) National standards for the practice of ecological restoration in Australia. Restor Ecol 24:S4–S32. doi: 10.1111/rec.12359

Meissen JC, Galatowitsch SM, Cornett MW (2015) Risks of overharvesting seed from native tallgrass prairies. Restor Ecol 23:882–891. doi: 10.1111/rec.12295

Meissen JC, Galatowitsch SM, Cornett MW (2017) Meeting Seed Demand for Landscape-Scale Restoration Sustainably: The Influence of Seed Harvest Intensity and Site Management. Ecoscience 24:145–155. doi: 10.1080/11956860.2017.1386482

Menz MHM, Dixon KW, Hobbs RJ (2013) Hurdles and Opportunities for Landscape-Scale Restoration. Science (80- ) 339:526–527. doi: 10.1126/science.1228334

Merritt DJ, Dixon KW (2011) Restoration Seed Banks--A Matter of Scale. Science (80- ) 332:424–425. doi: 10.1126/science.1203083

Nellemann C, Corcoran E (2010) Dead Planet , Living Planet. Biodiversity and ecosystem restoration for sustainable development. United Nations Environment Programme, GRID-Arendal, Norway

Nevill PG, Cross AT, Dixon KW (2018) Ethical seed sourcing is a key issue in meeting global restoration targets. Curr Biol 28:R1378–R1379. doi: 10.1016/j.cub.2018.11.015

Palmer MA, Filoso S (2009) Restoration of Ecosystem Services for Environmental Markets. Science (80- ) 325:575–576. doi: 10.1126/science.1172976

Paparella S, Araújo SS, Rossi G, et al (2015) Seed priming: state of the art and new perspectives. Plant Cell Rep 1281–1293. doi: 10.1007/s00299-015-1784-y

Scott JM (1989) Seed coatings and treatments and their effects on plant establishment. Adv Agron 42:43–83

Senaratna T, Touchell D, Bunn E, Dixon K (2000) Acetyl salicylic acid (Aspirin) and salicylic acid induce multiple stress tolerance in bean and tomato plants. Plant Growth Regul 30:157–161. doi: 10.1023/A:1006386800974

Society for Ecological Restoration International Science & Policy Working Group (2004) The SER International primer on ecological restoration. Tucson

Stevens J, Chivers I, Symons D, Dixon K (2015) Acid-digestion improves native grass seed handling and germination. Seed Sci Technol 43:313–317. doi: http://dx.doi.org/10.15258/sst.2015.43.2.19

Stevens J, Senaratna T, Sivasithamparam K (2006) Salicylic acid induces salinity tolerance in tomato (Lycopersicon esculentum cv. Roma): Associated changes in gas exchange, water relations and membrane stabilisation. Plant Growth Regul 49:77–83. doi: 10.1007/s10725-006-0019-1

Taylor AG, Paine DH, Suzuki N, et al (2004) Coating technologies for seed treatment applications. Acta Hortic 631:49–54

Turner SR, Pearce B, Rokich DP, et al (2006) Influence of polymer seed coatings, soil raking, and time of sowing on seedling performance in post-mining restoration. Restor Ecol 14:267–277. doi: 10.1111/j.1526-100X.2006.00129.x

UNEP-WCMC, IUCN (2016) Protected Planet Report 2016. Prot Planet Rep 2016 84

Van Bavel J (2013) The world population explosion: causes, backgrounds and projections for the future. Facts, views Vis ObGyn 5:281–91. doi: 10.1007/s00300-006-0172-z

Vander Mijnsbrugge K, Bischoff A, Smith B (2010) A question of origin: Where and how to collect seed for ecological restoration. Basic Appl Ecol 11:300–311. doi: 10.1016/j.baae.2009.09.002

Waters C, Whalley W, Huxtable C (2017) Microlaena stipoides (Microlaena or Weeping grass). In: Grassed up - Guidel. Reveg. with Aust. Nativ. grasses. http://www.dpi.nsw.gov.au/agriculture/pastures-and-rangelands/rangelands/publications-and-information/grassedup/species/microlaena

Western D (2001) Human-modified ecosystems and future evolution. Proc Natl Acad Sci 98:5458–5465. doi: 10.1073/pnas.101093598

Wijdeven SMJ, Kuzee ME (2000) Seed Availability as a Limiting Factor in Forest Recovery Processes in Costa Rica. Restor Ecol 8:414–424. doi: 10.1046/j.1526-100x.2000.80056.x

# 1. **Seed coating: science or marketing spin?***

## 1.1. Abstract

Seed coating is the practice of covering seeds with external materials to improve handling, protection and, to a lesser extent, germination enhancement and plant establishment. With an annual value exceeding one billion dollars, this technology is mostly the preserve of the private research sector, with few links to the scientific community. Here we analyse the science and industry of seed coating and its contribution to seed establishment and plant performance. We posit that a closer collaboration between academia and industry is critical to realising the potential of seed coating both as a tool for enhancing plant establishment in the face of the challenges posed to agricultural systems and to propel the multi-billion-dollar global push for ecological restoration of degraded ecosystems.

## 1.2. What is seed coating and why is it done?

Almost a century old (Kaufman 1991), the practice of seed coating has become the mainstay for many of the world's horticultural and crop industries with a global value estimated at $53.76 billion/year in 2014 [i].

Seed coating is the process of applying exogenous materials to the surface of the natural seed coat. This practice is used to modify the physical properties of seed (Kaufman 1991; Avelar et al. 2012), and for the delivery of active ingredients. The physical modification of seed aims to improve seed handling through standardization of seed weight and size (Halmer 2008). In some cases, where the aim is to reduce friction and improve flowability, the alteration of seed morphology is minimal, but for small (e.g. begonia, tobacco), expensive, or morphologically uneven seeds, a thicker

---

* This chapter was published on February 2017: Pedrini S, Merritt DJ, Stevens J, Dixon K (2017) Seed Coating: Science or Marketing Spin? Trends in Plant Science. 22:106–116 . doi: 10.1016/j.tplants.2016.11.002

**Authors' contribution**: Simone Pedrini and Kingsley Dixon conceived the study. Simone Pedrini developed the database for the organisation and analysis of the literature, collected and interpreted the data, and wrote the manuscript. Kingsley Dixon, Jason Stevens and David Merritt provided editing and revision to the manuscript. Simone Pedrini and Kingsley Dixon coordinated the publication process.

coverage is often applied. The artificial coat is frequently used as a carrier for a variety of active ingredients.

With the introduction of seed coating technology in developing countries, the global market for the materials alone (polymers, colourants and bulking agents) that are used in seed coating is expected to reach $1.63 billion/year by 2020 [ii].

Currently, seed coating is performed almost exclusively on crop and vegetable varieties and the application of this technology to wild plant species for ecological restoration, whose market is estimated at $ 18 billion/year (Menz et al. 2013), has been rarely explored by the private companies involved in the development and application of seed coatings.

## 1.3. The case for expanding seed coating technologies to wild species

Seed coating technologies have been developed on crop and vegetable species for the most part, and, to a lesser extent, on seeds of turf grass, pasture and flowers.

The application of coatings to native species for ecological restoration has received little attention, with only sporadic evaluation in the scientific literature (Madsen et al. 2012, 2014; Williams et al. 2016), and, so far, this field remains overlooked by the major agrochemical and seed technology companies. Yet seed is fundamental to meeting global restoration targets such as the rehabilitation of 150 million ha of degraded land by 2020 which is one of the United Nations sustainable development priorities [iii].

With the success rate of seedling establishment in restoration programs generally less than 10% (James et al. 2011; Merritt and Dixon 2011) the scope for seed improvement of native plant species is vast.

There is a pressing need for new approaches to seed-based restoration and seed coating technologies could be key to improving seedling establishment (Liu et al. 2010), plant growth (Madsen et al. 2012), and the restoration efficacy of native seed, most of which is collected from wild sources and represents a finite resource not to be wasted (Turner et al. 2006).

If the UN goals for ecological restoration are to be met, it is time to forge enhanced links between private and public seed technology research efforts. The development and commercialization of seed coating solutions for the emerging restoration ecology market could represent a major area of business for seed technology and agrochemical corporations, improve their environmental credentials, and provide new opportunities to deliver on their stated social obligations.

## 1.4. Equipment, materials and biological effects

It is common for studies of seed coating published in the scientific literature to have outsourced the coating process to private seed companies (Box 1: Seed coating equipment), meaning the specific details of the application technologies and materials are not disclosed.

Where academia has performed seed coating independently (without industry participation), simplified small-scale approaches (e.g. laboratory mixers or shakers, manual coatings, seeds shaken in plastic bags, or experimental technologies such as liquid nitrogen (Pilar-Izquierdo et al. 2012), seed moulding (Sikhao et al. 2015), and seed extrusion (Madsen et al. 2014)) in preference to the industrial standards (Figure 1.1) (Supplemental Information online, Figure S.1 and Figure S.2). The dissimilarity in the equipment employed, and the difficulty in accessing information on materials and methods, are indications of the scarce transparency of industry. This lack of disclosure limits the capacity for independent scientific evaluation of the improvements delivered by seed coatings, and potentially compromises the critical analytical processes that could improve the understanding and adoption of seed enhancement technologies.

Nevertheless, the materials used in the seed coating process can be broadly categorized according to their function as **binders** (see Glossary), **fillers** and active ingredients (Box 2: Seed coating type and materials).

*Box 1: Seed coating equipment*

The rotating pan was the first machine employed for seed coating and derived from a patent lodged at the end of the 19$^{th}$ century (William E. Upjohn 1885). It is composed of a round pan, usually inclined, on a rotating motorised pivot. Seeds are placed inside the pan and while the pan is rotating, liquids are applied with a spray nozzle, and powders are added through a hopper or by manual dusting. Rotating pans are mostly

used to form pellets and rely on a slow rotating motion (5-35 rpm depending on diameter) (Scott 1989) and the gradual addition of materials to increase pellet size (Scott et al. 1997). The friction of seeds tumbling on each other is responsible for the spherical shapes produced and acts to smooth the external pellet surface. The process is followed by size-sorting with sieves, and then drying (Halmer 2000). A low-cost alternative to the rotating pan used in some studies is a cement mixer (Hathcock et al. 1984); this may have application in developing countries with limited resources.

The fluidised or spouted bed apparatus, originally conceived in 1970 for drying solids (Harkreader 1970), was first adapted for seed coating in 1975 (Hinkes 1975). This apparatus is cylindrical, with seeds subjected to a constant sub-floor air flow that is adjusted to enable the seeds to remain buoyant in the air (Halmer 2000). A spray nozzle atomizes the coating liquid or slurry towards the suspended seed mass. This process is used for film coating and superficial encrusting but is not feasible for pelleting.

A machine that allows for both film coating and pelleting is the rotary coater or rotor-stator. It is composed of a cylindrical drum, with a concave disk at the base, whose rotation causes the seed mass to move in a regular flow along the walls of the drum. A smaller rotating disk that is responsible for the atomization and projection of liquid or slurry to the rotating seed mass is usually attached to the drum lid and suspended in the middle of the drum (Halmer 2008; Madsen et al. 2014).

These three systems are standard in the seed coating industry and are integrated into many seed treatment plants to allow for automated procedures and for continuous batch applications. Due to the high number of variables involved, including the material combinations, machine tuning, and seed morphological differences, it is not always feasible to rely entirely on automated systems and the “art and craft” of an experienced operator is often required to ensure the quality of the final product (Halmer 2000).

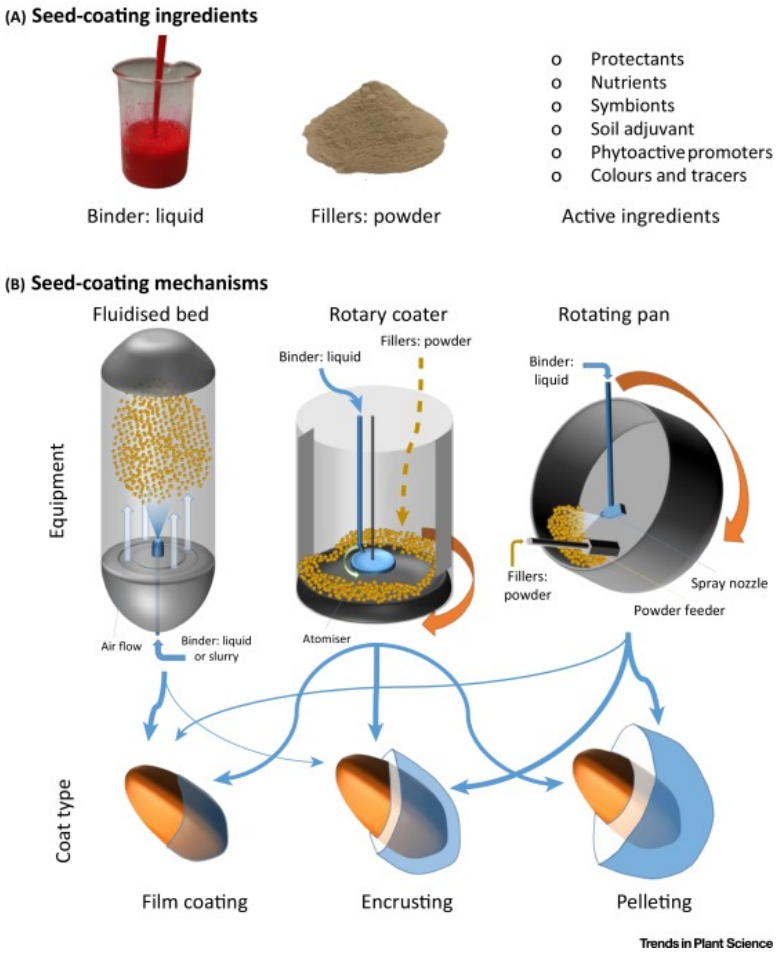



*Figure 1.1: Seed coating ingredients, equipment and coat type. (A) Seed coating ingredients (B) seed coating mechanism. The orange arrows on the equipment represent the motion of the moving components; the equipment parts and arrows coloured in blue illustrate the method of delivery of the liquid binding agent; and the gold arrows show where the fillers/powders are applied. Active ingredients can be added either mixed with the liquid, with the powder, or independently. The blue arrows indicate which coatings can be achieved from each of the equipment types. The weight of these blue arrows represents the effectiveness of any particular machine for producing the various types of coatings.*

*Box 2: Seed coating type and materials*

Seed coatings are categorized according to their physical characteristics. Although the nomenclature used in the literature is not consistent, the terminology most employed and recognized among industry and academia is based on the weight, size and sorting properties of the coated seeds. The basic coating treatment is **film coating,** where a thin layer of external material (usually less than 10% of seed weight) is applied. Where seed weight is increased up to 100-500% (depending on seed morphology), the procedure is described as **encrusting,** and is defined as such as long as the original shape of the seed is still evident (Halmer 2000; Gregg and Billups 2010). Where the amount of external material makes it impossible to discriminate the initial seed shape

(the result usually being a spherical shape), the process is named **pelleting** (Taylor et al. 1998). While film coated and encrusted seed are discerned by weight, pellets are sorted by diameter.

The structural materials employed in seed coating are categorized into binders and fillers**.** Binders are polymers of both natural and synthetic origin that provide adherence and cohesion of material onto the seed and the retention of active ingredients. They are usually applied in liquid form (in water or solvents), and when dried the dissolved monomers are re-joined in long polymeric chains forming a continuous film surrounding the seed, binding particles, and chemicals. Different layers of polymers can be applied at different stages of the coating process, some carrying treatments, and others providing a buffer to avoid direct contact between the "active layers" and the seed, the external environment, or other active layers.

In the majority of published scientific papers, seed coating has been undertaken with commercial binders of undisclosed composition. However, the most commonly reported binders are methylcellulose, polyethylene glycol, chitosan, polyvinyl alcohol, ethyl cellulose, polyvinyl acetate and gum arabic.

Pelleting and encrusting processes require the addition of a bulking agent that allows physical modification. This process is performed with either a single material, or a combination of multiple materials.

Fillers are usually inert powders like bentonite, calcium carbonate, talc, and diatomaceous earth, sand and wood dust (supplemental information Figure S.2 and Figure S.3).

The physical and chemical proprieties of the different powders, in combination with the binders, provide a wide variety of possible mechanical and biological outcomes for coatings. Particle size distribution Scott (Scott 1989), for example, strongly affects the pellet behaviour; small particles provide higher physical resistance but limited gas and water exchange (Grellier et al. 1999), whereas larger particles increase porosity, but reduce mechanical integrity and coat resilience.

Binders and fillers must be compatible with active compounds, and not adversely impact the ability of a seed to germinate and grow.

According to the characteristics of the natural seed coat (testa), applied compounds can be dissolved and transmitted into the seed via imbibing water, or if the testa is impermeable to those substances, through the uptake by the emerging radicle and root system (Salanenka and Taylor 2008, 2011).

### 1.4.1. *Protectants*

The most commonly reported active ingredients in coatings include fungicides, pesticides, insecticides, nematicides, predator deterrents and herbicides (box 3). The use of **protectant** treatments at best only slightly promotes germination and emergence, and sometimes in fact negatively affects the rate of germination (Yang et al. 2014). However, protectant compounds do usually benefit plant growth and yield through reducing predation and infection by pathogens (supplemental information online Figure S.4).

Despite these benefits, sometimes the protectants employed in coatings have negative off-target environmental impacts. For example, neonicotinoids, the most widely employed insecticidal compounds (Jeschke et al. 2010) in crop seed coatings, have been shown to cause a detrimental effect on wild bee diversity and distribution (Rundlöf et al. 2015), with indirect impacts on honey bee health (Alburaki et al. 2015). Moreover fungicidal and insecticidal coating products have indirect effects on the soil seed bank, potentially interfering with agroecosystem processes (Smith et al. 2016).

Further commitment is required by the agrochemical companies in developing new seed treatments to reduce off-target ecological impacts. Collaboration with the scientific community could help improve the testing of the efficacy of such products, and a broader ecological approach will allow for a more comprehensive assessment of potential biological impacts.

### 1.4.2. *Nutrients*

Where studies have evaluated nutrient amendments in seed coatings, their effects on germination, growth, and yield are usually positive (Supplemental Information Figure S.5). However, although the application of macronutrients like phosphorus (Peltonen-Sainio et al. 2006) and potassium (Tavares et al. 2013) improve growth and yield, there is the possibility of deleterious impacts on germination and emergence (Peltonen-Sainio et al. 2006; Mašauskas et al. 2008) caused by nutrient-induced osmotic stress

(Scott 1989). Most nutrient amendments have instead focused on the delivery of micronutrients such as, boron (Rehman et al. 2012; Rehman and Farooq 2013), copper (John et al. 2005; Wiatrak 2013a), manganese (Wiatrak 2013b), molybdenum (Hara 2013), and zinc (Oliveira et al. 2014; Adhikari et al. 2016). These amendments have been used to compensate for soil deficiencies in these trace elements (Farooq et al. 2012). The integration of seed biology, plant physiology, and soil science with a broader collaboration with the seed industry could optimize the use of seed coating as a way of delivering nutrients, ultimately allowing for the cultivation of varieties with predefined micronutrient requirements tailored to soil types with different trace elements deficiencies.

### 1.4.3. *Symbionts*

The integration of symbiotic organisms into coatings most commonly involves rhizobia for the inoculation of legumes (Deaker et al. 2004) leading to improvements in seedling growth and, to a lesser extent, germination (Supplemental Information Figure S.6). However, the incorporation of inocula in an artificial seed coat can result in loss of microbial viability, with coated seeds unable to be stored for extended periods (Scott 1989).

The artificial seed coat is usually a hostile environment for the rhizobia, mostly due to osmotic (John et al. 2010) and desiccation stress (McIntyre et al. 2007), and when protectant compounds are present, their biological activity could pose a threat to the survival of symbiotic bacteria (Scott 1989). The evaluation of more "rhizobia friendly" coating formulations, along with the selection of desiccation resistant bacteria, could improve symbiotic organism survival and the useful storage life.

### 1.4.4. *Soil adjuvants*

Soil hydrophilic materials or hydro absorbers (**hydrogels**) are the most commonly used compounds in seed coatings for their inherent capability to attract and retain water in proximity to the seed (Mangold and Sheley 2007; Gorim and Asch 2012; Serena et al. 2012). Another strategy to increase water availability to seeds and seedlings in water repellent soil is to apply a soil surfactant within the seed coating material (Honglu and Guomei 2008; Madsen et al. 2013).

*Box 3. Science and industry of seed coating active ingredients*

Using the last published major review on seed enhancement in 1998 (Taylor et al. 1998) as the starting point, we analysed those publications examining seed coating technologies since that time, evaluating in all 145 refereed publications. With additional research into the web-published, trademarked and registered seed coating materials developed by the main agrochemical and seed technology companies, we identified 191 products that have been used in coatings.

A comparison between the academic and industrial application of seed coating technologies shows some similarity in the kind of species tested; that is, mostly crop and vegetable varieties (Figure 1.2). However, where the use of active ingredients has been reported, some differences between the public and private research sectors start to appear. Coating products employed by industry contain mostly colours and protectants, with limited records of the use of inoculants, nutrients, and phytoactive promoters. In contrast, published scientific literature reports mostly on protective compounds, with inoculants and other ingredients also commonly reported.

Moreover, the seed industry claims benefits of coatings focussed on those products that deliver cosmetic outcomes and enhanced mechanical proprieties, such as improving flowability and handling, product adherence, and reduction of the **dust-off** effect. This diverges from the academic research that concentrates mostly on the germination, emergence and growth responses of coated seeds. It is clear that the scientific and industrial research sectors have had limited interaction and that the development of coating technologies has followed separate paths.

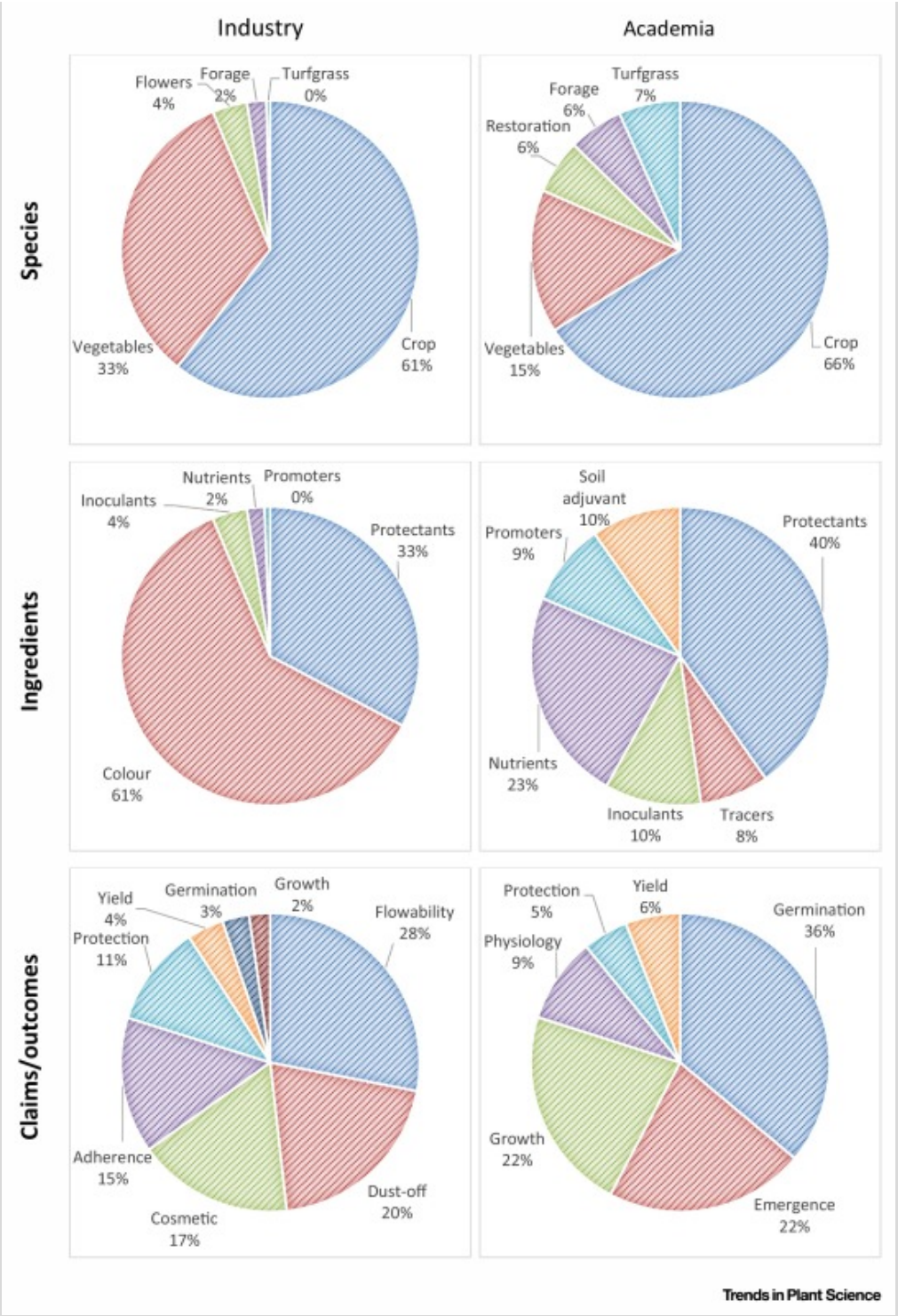



*Figure 1.2: Differences among seed industry and academia in terms of species evaluated, seed coating ingredients employed and outcomes or claims effects of the coating treatment.*

Some coatings have also been used to delay germination through influencing water absorption, in a sense creating an artificial dormancy. This kind of coat inhibits germination when climatic conditions are not optimal (Archer and Gesch 2003), and

usually provides protection from pathogens, fungi and predators (Johnson et al. 1999). This approach allows for early planting, relying on the coat to trigger the germination process when suitable conditions arise (Stokes 2001; Vyn and Murua 2001; Archer and Gesch 2003) and thereby can improve seedling emergence in no-tillage soil (Gesch et al. 2012). The delay is usually achieved through temperature activated polymers that regulate water uptake at predefined temperature thresholds.

### 1.4.5. *Phytoactive promoters*

Phytoactive promoters comprise a range of compounds that, once incorporated into seed coatings, potentially stimulate germination, promote growth and improve stress resistance (Table 1.1).

The limited number of promoters considered or disclosed in publications and in commercial products show how those potentially highly beneficial compounds have been mostly overlooked. However, the advantages of **phytoactive compounds** in the few cases where they have been tested (Table 1.1), suggests potential benefits for large-scale improvement in crop performance. Further investigations of these compounds are needed to better understand the efficiency of delivery through the seed coat. The use of promoters has the potential to improve: seedling and plant vigour; resistance to biotic/abiotic stresses; performance under water, salinity, and temperature stress conditions. Innovation in the deployment of phytoactive compounds via seed coats and pellets could be a key part of making farming possible in degraded areas or those areas adversely affected by climatic changes

### 1.4.6. *Tracers and colours*

The incorporation of fluorescent dyes and magnetic powder into coatings (Guan et al. 2013) has been developed to improve the traceability of seed batches through the supply chain, thereby limiting the risk of mislabelling and misplacement and allowing for the detection of counterfeit seed batches.

Colours are the most commonly employed amendment used in seed coating processes (box 3). United States federal regulation of seed treatments [iv] mandates the use of artificial non-natural colours for seed treated with pesticide to highlight to seed-users the presence of harmful compounds and reduce the risk of inadvertent consumption. Moreover, colour in seed coatings can help companies and users to differentiate seed of

different origin, variety or treatment; it also facilitates the identification of seed in the field during sowing operations for ease of calibration of seeding equipment and for controlling seeding efficiency. Furthermore, the use of brightly coloured coats, in combination with layers of inert material and binders, has been found to limit the predation of corn seed by birds (Almeida et al. 2010). Notwithstanding these applications for the use of colour, there is a notably strong focus by industry on colour as an ingredient in coatings (Box 3); clearly the cosmetics of coatings (Taylor et al. 1998; John et al. 2005; Jamieson 2008) provide a marketing boon for seed companies.

## 1.5. The market for coated seed

Though many private companies have been working on the improvement and commercialisation of seed coating technologies and coated seeds, much of the global market is nowadays controlled by six transnational agrochemical companies: Bayer Crop Science (Germany), Syngenta (Switzerland), Monsanto (USA), BASF (Germany), DuPont-Pioneer (USA) and Dow (USA). The core business, and research focus, of these enterprises lies mainly in the development of new plant varieties through breeding, genetic engineering and the improvement of chemical and biological protectants [v]. Seed coating technologies represent an effective means of applying these compounds onto selected seeds to combine the effect of genetically enhanced varieties with protectants. Smaller enterprises, such as Incotec (Netherland/United Kingdom) and Germains (United Kingdom) are dedicated to seed technology development and are major innovators in the seed coating market (Supplemental information Figure S.7). They are specialised in the physical-mechanical aspects of seed coating with a focus on improving the retention of active ingredients, handling proprieties and the overall efficiency of seed delivery (sowing). However, the information provided by companies through websites, marketing campaigns, and press releases are not peer-reviewed nor independently tested and should, therefore, be considered carefully and critically.

*Table 1.1: Effects of Phytoactive compounds*

| SPECIES | CONDITION | PHYTOCTIVE COMPOUNDS | RECORDED[a] | RESULTS[b] | REFERENCE |
|---|---|---|---|---|---|
| *Zea mays* | Lab – Chill stress | 0.5 g/kg salicylic acid | Germination F | =+ | (Guan et al. 2015) |
| | | | Growth F | + | |
| | | 5g/kg salicylic acid | Germination F | - | |

| | | | | | |
|---|---|---|---|---|---|
| | | | Growth F | - | |
| | | 0.5 and 5 g/kg salicylic acid + Hydrogel | Germination F | + | |
| | | | Growth F | + | |
| *Nicotiana tabacum* | Lab – Drought stress | 0.5 g/kg salicylic acid | Germination F | = | (Guan et al. 2014) |
| | | | Growth F | = | |
| | | 1.0 g/kg salicylic acid | Germination F | + | |
| | | | Growth F | + | |
| | | 1.5 g/kg salicylic acid | Germination F | - | |
| | | | Growth F | - | |
| | | 0.5, 1.0 and 1.5 g/kg salicylic acid + hydrogel | Germination F | + | |
| | | | Growth F | + | |
| *Zea mays* | Lab – Optimal | 8 mmol/l hydrogen peroxide | Germination R and F | =+ | (Lizárraga-Paulín et al. 2013) |
| | Glasshouse - Optimal | 8 mmol/l hydrogen peroxide | Emergence F | =+ | |
| | | | Growth F | =+ | |
| *Oryza sativa* | Field trial | 1000 mg/l gibberellic acid | Emergence F | + | (Gevrek et al. 2012) |
| | | | Growth F | + | |
| | | | Yield | + | |
| *Festuca arundinacea* | Field trial | commercial growth stimulant | Emergence F and R | = | (Richardson and Hignight 2010) |
| *Poa pratensis* | Field trial | commercial growth stimulant | Emergence F and R | = | |
| *Oryza sativa* | Lab – optimal | 0.002 % gibberellic acid | Germination F | =+ | (Zeng and Shi 2009) |
| | | | Growth F | =+ | |
| | Field trial | 0.002 % gibberellic acid | Emergence F | =+ | |
| *Capsicum annum* | Lab – optimal | 50mg/l gibberellic acid, 90mg/l kinetin, 50 mg/l auxin (commercial mix) | Germination F | = | (Diniz et al. 2009) |
| | | | Growth F | = | |
| *Oryza sativa* | | abscisic acid 10 mg/l | Injury resistance | + | |

| | | | | | |
|---|---|---|---|---|---|
| | Lab and field – chill stress | | Root vigor | + | (ZHANG et al. 2007) |
| | | | Chlorophyll and sugar | + | |
| *Lactuca sativa* | glasshouse | gibberellic acid, kinetin, auxin – (commercial mix) | Emergence F and R | + | (Diniz et al. 2006) |
| *Zea mays* | filed trial | commercial bio stimulant | Yield | = | (Tweddell et al. 2000) |

[a]*F=final, R=rate.*

[b]*Due to the diversity of experimental approaches and heterogeneity of the data a qualitative scale was designed to represent the results based on the statistically significant difference between the coated seed compared with untreated control. To account for the outcomes, when different coating treatments were tested, the result has been reported on a scale that incorporates the potential combinations: negative (-), negative or neutral (-=), neutral ( =), neutral or positive (=+) and positive (+).*

## 1.6. Marketing benefits

Seed companies stress the importance of a coloured, coated seed as evidence of seed quality. End-users now expect their seed to be in a coloured and/or bulked state, and the supply of such seed is now the industry norm. Although the modification of seed size through **encrusting** and **pelleting** improves seed handling and allows for the application of more active ingredients, these build-up processes are portrayed by companies, and perceived by customers, as high-tech and somewhat crucial for enhancing seed establishment and plant performance. But the lack of independent testing to support these claims, and the difficulty in accessing company information on the pelleting processes and compositions, raises the question: *Is all that inert material necessary?* After all, the main goal of any commercial enterprise is to generate revenue, maximizing profit, and the exploitation of such a powerful marketing tool that is afforded by coating is understandably driven by market expectations.

But to what degree do seed companies bulk seed to maximise profit, demanding premium prices for small amount of seeds pelleted in large quantities of relatively inexpensive, inert materials containing few active ingredients?

The global seed market is progressing toward consolidation (Howard 2009) with the merging of the major transnationals including the ongoing acquisition of Syngenta by ChemChina [vi], and the take-over of Monsanto by Bayer [vii]. Such a trend increases the risk of oligopoly or even monopoly of the seed enhancement market with company behaviour no longer kept in check by the pressure of competitors (Heijnen 2008).

Moreover, the many collaborations and connections recorded among companies (Howard 2009) suggest that a similar scenario might already be unfolding, with trade secrets and trademark barriers in place to conceal technological know-how that might not be as advanced or effective as customers, and society in general, are bound to believe. This has important consequences if seed coating technology is to deliver the crop benefits for feeding an ever-increasing global population, particularly in economically disadvantaged countries.

## 1.7. Concluding remarks and future opportunities

This critique is aimed at increasing the transparency of seed companies, in the hope that the very active (and well-funded) industry research departments will consider sharing and disclosing methodological and experimental results, to back up commercial claims with verifiable data and to promote the overall advancement of seed coating technologies.

The research foci of academia and industry in this field have historically diverged, with private companies improving the mechanical proprieties of seed coatings, and the scientific community mostly focussed on evaluating the efficiency and impacts of active ingredients. Their integration could be highly beneficial for both the private sector and academia.

For example, a recently developed framework for the analysis of wild seed recruitment identifies the critical plant life-stage transition(s) that contribute most to establishment failure (James et al. 2011). This approach is a powerful tool that could be easily adapted to crop farming, directing seed coating solutions that maximise seedling establishment, plant growth, and yield.

The disclosure by industry of the innovative, and already optimised materials used in coating (in terms of mechanical performance) would allow researchers to improve seed coatings in their area of expertise, rather than engaging significant effort in the

fine-tuning of those materials and processes that have already been developed within the private sector.

This could then increase the interest and engagement of the scientific community in seed coating technologies, resulting in more studies, publications and breakthroughs that could subsequently be adopted by industry. For example, further research and development of the use of phytoactive promoters in coatings, largely over-looked to date, could improve seed germination, seedling establishment, and stress resistance, potentially reducing the need for environmentally harmful protectants and increasing food security in spite of the threats posed by climate warming, pollution (Tai et al. 2014) and drought (Daryanto et al. 2016) to agricultural systems (see Outstanding Questions).

The field of endeavour that could benefit the most from the application of coating technologies is ecological restoration. At present seed availability, high cost (Merritt and Dixon 2011) and low seed establishment rate (Menz et al. 2013) represent serious limitations to the effective use of native seed. Advanced seed technologies such as seed coating could be a key step in achieving cost-effective ecosystem recovery at the planetary scale, while providing the benefit of new business opportunities for seed companies.

## 1.8. Glossary

**Binder:** a liquid with adhesive proprieties used to provide structural support and retention of active ingredients.

**Dust-Off**: the release of dusty material from the surface of treated or coated seeds as a result of mechanical stresses and frictions during handling and sowing.

**Encrusting:** a coating process whereby powder and liquid binders are applied to the seed, causing a significant increase in weight and volume without altering the original seed shape.

**Filler**: a powdery, inert material used to increase seed shape and size.

**Film coating:** the application of a thin layer of material onto the seed surface. Weight gain, shape, and size modification of the seed is very limited.

**Hydrogels**: polymers with hydrophilic structures that allow for the absorption and retention of a large amount of water.

**Pelleting:** the application of sufficient material to significantly modify seed morphology into a flowable spherical or ovoid shape; the most conspicuous of the coating treatments available**.**

**Protectants:** various active ingredients aimed at protecting the seed from seed or soil-borne diseases and threats such as nematodes, bacteria, fungal infections, predator insects and competing plant species.

**Phytoactive compounds**: active ingredients that promote germination, enhance seedling emergence, survival and growth, and provide resistance to biotic and abiotic stresses.

## 1.9. Resources

[i] http://www.marketsandmarkets.com/PressReleases/seed.asp

[ii] http://www.marketsandmarkets.com/Market-Reports/seed-coating-materials-market-149045530.html

[iii] http://vote.riodialogues.org/

[iv]https://www.law.cornell.edu/cfr/text/40/153.155

[v] http://news.agropages.com/News/NewsDetail---14163.htm

[vi] http://www.reuters.com/article/us-syngenta-m-a-chemchina-idUSKCN12P186

[vii] http://www.bloomberg.com/news/articles/2016-09-14/bayer-clinches-monsanto-deal-with-fourth-offer-of-66-billion

## 1.10. Bibliography

Adhikari T, Kundu S, Rao AS (2016) Zinc delivery to plants through seed coating with nano-zinc oxide particles. Journal of Plant Nutrition 39:136–146

Alburaki M et al. (2015) Neonicotinoid-Coated Zea mays Seeds Indirectly Affect Honeybee Performance and Pathogen Susceptibility in Field Trials Rodriguez-Saona, C, editor. PLOS ONE 10:e0125790

Almeida A De, Couto HTZ Do, Almeida ÁF De (2010) Are camouflaged seeds less attacked by wild birds? Scientia Agricola 67:170–175

Archer DW, Gesch RW (2003) Value of Temperature-Activated Polymer-Coated Seed in the Northern Corn Belt. Journal of Agricultural and Applied Economics 35:625–673

Avelar SAG et al. (2012) The use of film coating on the performance of treated corn seed. Revista Brasileira de Sementes 34:186–192

Daryanto S, Wang L, Jacinthe P-A (2016) Global synthesis of drought effects on cereal, legume, tuber and root crops production: A review. Agricultural Water Management

Deaker R, Roughley RJ, Kennedy IR (2004) Legume seed inoculation technology - A review. In: Soil Biology and Biochemistry.Vol. 36 pp. 1275–1288.

Diniz KA et al. (2006) Incorporação de microrganismos, aminoácidos, micronutrientes e reguladores de crescimento em sementes de alface pela técnica de peliculização. Revista Brasileira de Sementes 28:37–43

Diniz KA et al. (2009) Sweet pepper seed responses to inoculation with microorganisms and coating with micronutrients, aminoacids and plant growth regulators.

Farooq M, Wahid A, Siddique KHM (2012) Micronutrient application through seed treatments: a review. Journal of soil science and plant nutrition 12:125–142

Gesch RW, Archer DW, Spokas K (2012) Can using polymer-coated seed reduce the risk of poor soybean emergence in no-tillage soil? Field Crops Research 125:109–116

Gevrek MN, Atasoy GD, Yigit A (2012) Growth and Yield Response of Rice ( Oryza sativa ) to Different Seed Coating Agents. International Journal of Agriculture & Biology 14:826–830

Gorim L, Asch F (2012) Effects of Composition and Share of Seed Coatings on the Mobilization Efficiency of Cereal Seeds During Germination. Journal of Agronomy and Crop Science 198:81–91

Gregg BBR, Billups G (2010) Seed coating and pelletizing. In: Seed Conditioning, Volume 2. Science Publishers, Enfield, NH pp. 818–834.

Grellier P, Riviere LM, Renault P (1999) Transfer and water-retention properties of seed-pelleting materials. European Journal of Agronomy 10:57–65

Guan Y et al. (2014) An Enhanced Drought-Tolerant Method Using SA-Loaded PAMPS Polymer Materials Applied on Tobacco Pelleted Seeds. The Scientific World Journal 2014:9

Guan Y et al. (2015) "On-Off" Thermoresponsive Coating Agent Containing Salicylic Acid Applied to Maize Seeds for Chilling Tolerance. PLOS ONE 10:e0120695

Guan Y et al. (2013) The Novel Approach to Enhance Seed Security: Dual Anti-Counterfeiting Methods Applied on Tobacco Pelleted Seeds Niedz, RP, editor. PLoS ONE 8:1–9

Halmer P (2000) Commercial seed treatment technology. In: Seed Technology and its biological basis. I. Black, M & Bewley, JD, editors. Sheffield Academic Press, Sheffield pp. 257–283.

Halmer P (2008) Seed Technology and Seed Enhancement. Acta Horticulturae 771:17–26

Hara Y (2013) Improvement of Rice Seedling Establishment in Sulfate-Applied Submerged Soil by Application of Molybdate. Plant Production Science 16:61–68

Harkreader GG (1970) Fluid bed drying system - US 3494046 A.

Hathcock AL et al. (1984) Tall Fescue and Kentucky Bluegrass Response to Fertilizer and Lime Seed Coatings 1. Agranomy Journal 76:879–883

Heijnen S (2008) Stephan G. Hannaford: Market Domination!: The Impact of Industry Consolidation on Competition, Innovation, and Consumer Choice. Greenwood Publishing Group

Hinkes TM (1975) Seed coating process and product - US 3911183 A.

Honglu X, Guomei X (2008) Suspension Property of Gemini Surfactant in Seed Coating Agent. Journal of Dispersion Science and Technology 29:496–501

Howard PH (2009) Visualizing consolidation in the global seed industry: 1996-2008. Sustainability 1:1266–1287

James JJ, Svejcar TJ, Rinella MJ (2011) Demographic processes limiting seedling recruitment in arid grassland restoration. Journal of Applied Ecology 48:961–969

Jamieson G (2008) NEW PERSPECTIVES ON SEED ENHANCEMENT. Acta Horticulturae 782:143–150

Jeschke P et al. (2010) Overview of the Status and Global Strategy for Neonicotinoids (dagger). Journal of agricultural and food chemistry 1–7

John RP et al. (2010) Development of emulsion from rhizobial fermented starch industry wastewater for application as Medicago sativa seed coat. Engineering in Life Sciences 10:248–256

John SS et al. (2005) Seed Film Coating Technology for Maximizing the Growth and Productivity of Maize. Karnataka Journal of Agricultural Sciences 18

Johnson GA et al. (1999) Use of temperature-responsive polymer seed coating to control seed germination. In: Acta Horticulturae.Vol. 504 pp. 229–236.

Kaufman G (1991) Seed Coating: A tool for stand establishment; a stimulus to seed quality. HortTechnology 1:98–102

Liu Y, Horisawa S, Mukohata Y (2010) Effect of seed coating on plant growth and soil conditions: A preliminary study for restoration of degraded rangeland in the Qinghai-Tibetan Plateau, China. Grassland Science 56:145–152

Lizárraga-Paulín E-G et al. (2013) Maize seed coatings and seedling sprayings with chitosan and hydrogen peroxide: their influence on some phenological and biochemical behaviors. Journal of Zhejiang University. Science. B 14:87–96

Madsen MD et al. (2014) Improving Restoration of Exotic Annual Grass-Invaded Rangelands Through Activated Carbon Seed Enhancement Technologies. Rangeland Ecology & Management 67:61–67

Madsen MD et al. (2012) Agglomerating seeds to enhance native seedling emergence and growth. Journal of Applied Ecology 49:431–438

Madsen MD et al. (2013) Surfactant Seed Coating – a Strategy To Improve Turfgrass Establishment on Water Repellent Soils. In: International Symposium on Adjuvants for Agrochemicals. pp. 205–210.

Mangold JM, Sheley RL (2007) Effects of Soil Texture , Watering Frequency , and a Hydrogel on the Emergence and Survival of Coated and Uncoated Crested Wheatgrass Seeds. Ecological Restoration 25:6–11

Mašauskas V et al. (2008) Phosphorus seed coating as starter fertilization for spring malting barley. Acta Agriculturae Scandinavica, Section B - Plant Soil Science 58:124–131

McIntyre HJ et al. (2007) Trehalose biosynthesis in Rhizobium leguminosarum by. trifolii and its role in desiccation tolerance. Applied and Environmental Microbiology 73:3984–3992

Menz MHM, Dixon KW, Hobbs RJ (2013) Hurdles and Opportunities for Landscape-Scale Restoration. Science 339:526–527

Merritt DJ, Dixon KW (2011) Restoration Seed Banks--A Matter of Scale. Science 332:424–425

Oliveira S De et al. (2014) Tratamento de sementes de Avena sativa l. com zinco: qualidade fisiológica e desempenho inicial de plantas. Semina: Ciências Agrárias 35:1131

Peltonen-Sainio P, Kontturi M, Peltonen J (2006) Phosphorus seed coating enhancement on early growth and yield components in oat. Agronomy Journal 98:206–211

Pilar-Izquierdo MC et al. (2012) Barley seed coating with free and immobilized alkaline phosphatase to improve P uptake and plant growth. Journal of Agricultural Science 150:691–701

Rehman AU, Farooq M (2013) Boron application through seed coating improves the water relations, panicle fertility, kernel yield, and biofortification of fine grain aromatic rice. Acta Physiologiae Plantarum 35:411–418

Rehman AU et al. (2012) Optimizing the Boron Seed Coating Treatments for Improving the Germination and Early Seedling Growth of Fine Grain Rice. International Journal of Agriculture and Biology 14:453–456

Richardson MD, Hignight KW (2010) Seedling emergence of tall fescue and kentucky bluegrass, as affected by two seed coating techniques. HortTechnology 20:415–417

Rundlöf M et al. (2015) Seed coating with a neonicotinoid insecticide negatively affects wild bees. Nature 521:77–80

Salanenka Y a., Taylor AG (2011) Seedcoat permeability: Uptake and post-germination transport of applied model tracer compounds. HortScience 46:622–626

Salanenka YA, Taylor AG (2008) Seed Coat Permeability and Uptake of Applied Systemic Compounds. Acta Horticulturae 782:151–154

Scott JM (1989) Seed coatings and treatments and their effects on plant establishment. Advances in Agronomy 42:43–83

Scott JM, Blair GJ, Andrews AC (1997) The mechanics of coating seeds in a small rotating drum. Seed Science and Technology 25:281–292

Serena M et al. (2012) Turfgrass Establishment from Polymer-coated Seed Under Saline Irrigation. HortScience 47:1789–1794

Sikhao P et al. (2015) Development of seed agglomeration technology using lettuce and tomato as model vegetable crop seeds. Scientia Horticulturae 184:85–92

Smith RG et al. (2016) Evidence for indirect effects of pesticide seed treatments on weed seed banks in maize and soybean. Agriculture, Ecosystems and Environment 216:269–273

Stokes T (2001) Coating enables early planting. Trends in Plant Science 6:243

Tai APK, Martin MV, Heald CL (2014) Threat to future global food security from climate change and ozone air pollution. Nature Climate Change 4:817–821

Tavares LC et al. (2013) Potássio via recobrimento de sementes de soja: efeitos na qualidade fi siológica e no rendimento. Ciência Rural 43:1196–1202

Taylor AG et al. (1998) Seed enhancements. Seed Science Research 8:245–256

Turner SR et al. (2006) Influence of polymer seed coatings, soil raking, and time of sowing on seedling performance in post-mining restoration. Restoration Ecology 14:267–277

Tweddell RJ, Pelerin S, Chabot R (2000) A two-year field study of a commercial biostimulant applied on maize as seed coating. Canadian Journal of Plant Science 80:805–807

Vyn TJ, Murua M (2001) Polymer Seed Coatings : Sufficient Risk Reduction for Early Plant Corn ? In: 56th Annual Corn and Sorghum Research Conference. Purdue University pp. 1–11.

Wiatrak P (2013a) Effect of polymer seed coating with micronutrients on soybeans in southeastern coastal plains. American Journal of Agricultural and Biological Sciences 8:302–308

Wiatrak P (2013b) Influence of seed coating with micronutrients on growth and yield of winter wheat in southeastern coastal plains. American Journal of Agricultural and Biological Sciences 8:230–238

William E. Upjohn (1885) Process of making pills US312041.

Williams MI et al. (2016) Can biochar be used as a seed coating to improve native plant germination and growth in arid conditions? Journal of Arid Environments 125:8–15

Yang D et al. (2014) Microencapsulation of seed-coating tebuconazole and its effects on physiology and biochemistry of maize seedlings. Colloids and Surfaces B: Biointerfaces 114:241–246

Zeng D, Shi Y (2009) Preparation and application of a novel environmentally friendly organic seed coating for rice. Journal of the Science of Food and Agriculture 89:2181–2185

ZHANG H et al. (2007) Effect and Mechanism of Cold Tolerant Seed-Coating Agents on the Cold Tolerance of Early Indica Rice Seedlings. Agricultural Sciences in China 6:792–801

# 2. Protocol Development Tool (PDT) for seed encrusting and pelleting*

## 2.1. Abstract

Seed encrusting and pelleting are seed coating technologies that increase seed size and weight, improving handling, consistency in seed delivery and providing active ingredients for seed protection and enhancement. Though widely used for crop and vegetable seeds, with an estimated value of more than a billion dollars per annum globally, the know-how and methodologies are rarely disclosed by the commercial seed industry sector. As a result, it is difficult to reproduce specific seed coatings for research and comparative evaluation. For small seed producers, particularly the emerging native seed sector, seed enhancement technologies are either unavailable or rarely adopted due to their inaccessibility. Here we present the first fully disclosed Protocol Development Tool (PDT) for seed pelleting and encrusting. The PDT is customisable, applicable to a wide range of agricultural, horticultural and restoration purposes, and adaptable to suit a variety of seeds and coating materials. The PDT will allow researchers and seed suppliers to test and develop project-specific pelleting and encrusting methods within a standardised and replicable framework.

## 2.2. Introduction

Applied seed technologies have had a marked impact on the improvement of farming methods in the past century, and are now integral in crop and vegetable production systems worldwide. Seed coating, defined as the application of external material onto the outside of a seed, was developed in the early 20th century to reduce rodent and bird

---

* This chapter was published on July 2018: Pedrini S, Bhalsing K, Cross AT, Dixon KW (2018) Protocol Development Tool (PDT) for seed encrusting and pelleting. Seed Sci Technol 46:393–405. doi: 10.15258/sst.2018.46.2.21

**Authors' contribution:** Simone Pedrini conceived the study, developed the Protocol Development Tool and wrote the manuscript. Simone Pedrini and Khiraj Balshing tested the protocol development tool. Khiraj Balshing, Adam Cross and Kingsley Dixon provided editing and revision to the manuscript. Simone Pedrini coordinated the publication process.

predation of crop seeds and limit the effects of seed-borne diseases and fungal degradation (Mathre *et al*., 2001). The utility of seed coating was soon extended to the modification of seed size and weight to improve mechanical sowing efficiency, optimising seed deployment in agriculture through methods such as precision sowing (Scott, 1989; Taylor and Harman, 1990). Large-scale commercial use of seed coating and precision sowing began in the greenhouse transplant industry in the 1960s in Europe (Kaufman, 1991), followed shortly by the United States agricultural sector in the 1970s after the implementation of stricter legal requirements on the horticulture industry (Kaufman, 1991; Hill, 1999). Most agricultural and horticultural seeds currently available on the western market are now coated, and the technology is rapidly spreading to developing countries. The global market for coating and pelleting materials is forecasted to reach almost US $1.63 billion by 2020 (MarketsandMarkets, 2016).

Despite the significance and impact of seed coating technologies in the seed industry, most of the methodology and protocols remain 'commercial in-confidence' and the exclusive domain of major multinational seed supply and technology companies (Howard, 2009). A critical review of seed enhancements by Pedrini *et al.* (2017) showed that almost half of the material and methods employed in scientific studies were outsourced to private companies, and therefore undisclosed and unable to be reproduced or scientifically scrutinised and evaluated. Even when processes were reported, only a third of studies employed standard and scalable industry equipment and methods. This lack of transparency from the seed industry has limited the development and evaluation of seed enhancement technologies, hampering innovation in fields such as the rapidly expanding US $18 billion p.a. restoration sector (Menz *et al*., 2013), a major user of wild seeds (Merritt and Dixon, 2011).

We employ a 'reverse engineering' approach to unpack seed enhancement techniques and provide an open access, reproducible and customisable *Protocol Development Tool* (PDT). We provide a detailed description of the PDT along with two practical examples of its application, a customisable *pro forma* (Figure 2.1) and an excel table for data entry and analysis. This tool allows seed scientists and the seed industry to test and develop seed coating procedures tailored to species- and site-specific needs, and enable the incorporation of plant protective agents. Here, the PDT describes the physical, chemical and biological components of coated seeds, and extends the application of coating technologies beyond industrial crop and vegetable production to

industries such as seed-based ecological restoration and sustainable agriculture in developing countries.

## 2.3. Method description

Seed coating treatments are divided into three categories depending on the degree of seeds modification required. These include *film coating* (when a thin layer of material, usually in liquid form, is applied to the seed), *encrusting* (when seed size is increased, but the original shape of the seed is still evident) (Halmer, 2000) and *pelleting* (when the seed-unit is standardised to a near spherical shape and the original form of the seed is no longer discernible) (Taylor *et al*., 1998). As film coating is a relatively rapid and simple procedure, it is not included in the PDT we present; we use the term 'seed coating' to mean encrusting and pelleting techniques. Coated seeds, either pelleted or encrusted, are referred to as 'units'.

Seed coating requires at least two types of material:

1) Fillers, usually powdered inert inorganic (e.g. clays, talc) or organic (e.g. chitosan, wood dust) material used to increase unit size and weight.

2) Binder, usually delivered in liquid form, providing water to facilitate powder adhesion during the coating process and sustain the coating once dried.

Recently the seed industry has start using "coating blends" meaning that dry binder are delivered with the powder using water as the liquid component.

The complete list of equipment and required materials is provided online (http://www.arc-cmsr.org/download/PDT_EquipementMaterial.pdf).

The seed coating process is divided into six phases (Table 2.1), laid out in a *pro forma* to guide operators through the sequential process of developing a coating protocol (Figure 2.1, http://www.arc-cmsr.org/download/PDT_ProForma.pdf). This *pro forma* is preferably completed during the coating processphase, with data entered into the supplementary table that then auto-calculates the coating outcomes (http://www.arc-cmsr.org/download/PDT_Table.xlsx). The *pro forma* allows for reliable replication or modification of protocols, and the table provides a summary of outcomes to assist operators to further improve, adapt or scale the process. Across the *pro forma* and table, the input cells are colour coded to reflect required materials (blue for binder,

yellow for filler) and represent different phases (General Information [grey], Before Coating [green], Coating Process [orange], Wet Phase [blue], Dry Phase [yellow]). Additional Notes and Quality and Mechanical integrity tests are coded in white.

We recommend a copy of the *pro forma* (http://www.arc-cmsr.org/download/PDT_ProForma.pdf) is accessible while reading this article, as it will facilitate understanding of the procedure.

### 2.3.1. General Information recording phase (GI)

Prior to undertaking coating, general information such as the date, operator name and species of seeds to be coated should be recorded.

When just one kind of filler and binder are used, they are recorded here. If different materials are used for the different layers, they should be reported in the pelleting process section. The *pro forma* and table need to be customised accordingly. When "coating blends" are used instead of fillers and binders, they must be recorded, and, if necessary, the *pro forma* and table modified.

The filler used in the provided examples was diatomaceous earth sieved at 400 nm (The Green Life Soil Co., Perth, Australia) and the binder was hydroxyethyl cellulose (HEC) CELLOSIZE QP 09-L (DOW chemicals) at 2% w/w solution.

Tomato (*Lycopersicon esculentum* Mill. var. Tytanium) and the native Australian weeping grass (*Microlaena stipoides* (Labill.) R.Br. var. Griffin), were selected as test species to account for different techniques and potential fields of applications. Tomato was chosen to test seed pelleting for horticultural use and *M. stipoides* was chosen for its use in ecological restoration.

### 2.3.2. Before coating phase (BC)

In this phase, information such as the weight of seeds to be coated, time when the process commenced, initial weight of binder (BND) and powder (PWD) is recorded. It is important to prepare more material than necessary, to account for potential losses through wastage. The amount of material deployed onto the seed will be calculated by subtracting the material remaining at the end of the process. Examples use 10 g each of tomato and *M. stipoides* seeds.

## 2.3.3. Coating phase (CP)

This phase is divided into three stages for each successive layer of coating (Table 2.1).

*Table 2.1: Description of the six phases of the Protocol Development Tool and list of the equipment required at each phase.*

| | Phase | Stage | Process description | Required equipment |
|---|---|---|---|---|
| GI | General information | | Record information on date, operator name, species and material to be used | None |
| BC | Before coating | | Record initial weight of seed to be coated, powder and binder, and time when the process is started | Balance |
| CP | Coating phase | 1 | Deliver small quantities of binder and powder alternately until the desired amount of binder is delivered. Then weigh the coated seeds (units) and remaining powder. | Rotary coater<br>Air brush<br>Paint brush<br>Balance |
| | | 2 | Deliver binder and powder alternately until the desired amount of binder is delivered. Then sieve and weigh the units. This ends the encrusting process. | Rotary coater<br>Atomiser<br>Measuring spoon<br>Balance<br>Sieves |
| | | 3 | Deliver binder and powder alternately in larger quantities than stage two, until the desired unit size is achieved. Unit size is tested by sieving. This ends the pelleting process. | Rotary coater<br>Atomiser<br>Measuring spoon<br>Balance<br>Sieves |
| WP | Wet | | Weigh the fractions of desired, small and large units, and discard the last two fractions. Record the weights of leftover powder and binder, and the time when the process ended. Place units in the drying oven. | Sieves<br>Balance<br>Drying oven |
| DP | Dry | | Record weight of dried units, oven temperature and duration of the drying phase. | Balance |
| QM | Quality control | | Perform crush test and mechanical integrity test and report the results in the pro-forma. | Force gauge<br>Dissecting kit |

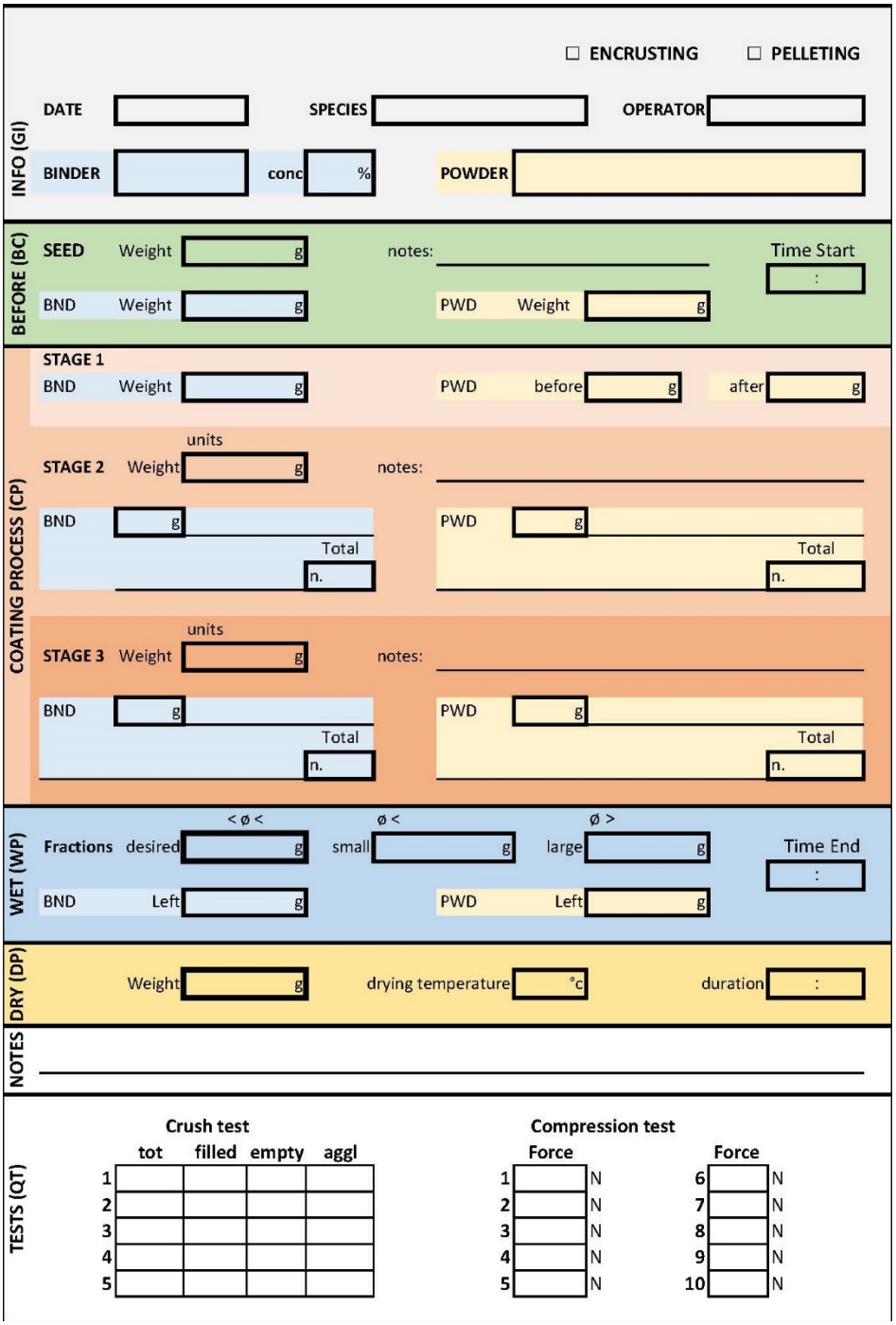



*Figure 2.1: Pro forma to be filled by the operator during encrusting or pelleting. It is divided into six phases. The information reported in the pro forma should then be entered in the excel table (www.arc-cmsr.org/download/PDT_Table.xlsx)*

In the examples, the pelleting and encrusting processes were performed in a laboratory bench-top rotary coater HR160 (Hoopman, Aalten, The Netherlands) (Figure 2.2),

however a rotating pan (or pan coater) could be used. Before starting it is important to make sure that the rotor (rotating dish) is moving freely inside the stator (static drum), and the air-line is active and connected to the coater Figure 2.2). If using fine powders we advise using a dust extractor placed over the coater and operators should wear a dust mask. For delivering the binder solution in Stage 1, (Figure 2.3) a compressed air propelled 0.7 mm air brush (Ozito Tools, Australia) was used. Compressed air for the rotary coater and air brush was provided by a 30 L, oil-free 2-piston air compressor (Sydneytools, Roseland, Australia). Air pressure was maintained at 0.3 and 0.4 MPa. The rotary coater was set in motion, seeds placed in the coater and a pre-determined quantity of binder placed in the paint cup of the air brush (in the examples, 10 g of binder for 10 g of seed). Rotation speed and inlet air were regulated to keep seeds within the coater.

The first step is to moisten the seeds using the air brush positioned 100-150 mm from the rotating seed mass. This allows the filler to adhere to the seeds. The viscosity of the liquid, usually dependant on binder concentration of the solution, affects the rate of delivery. More viscous liquid requires higher air pressure and longer air brush bursts. Binder viscosity should not exceed 100 centipoise.

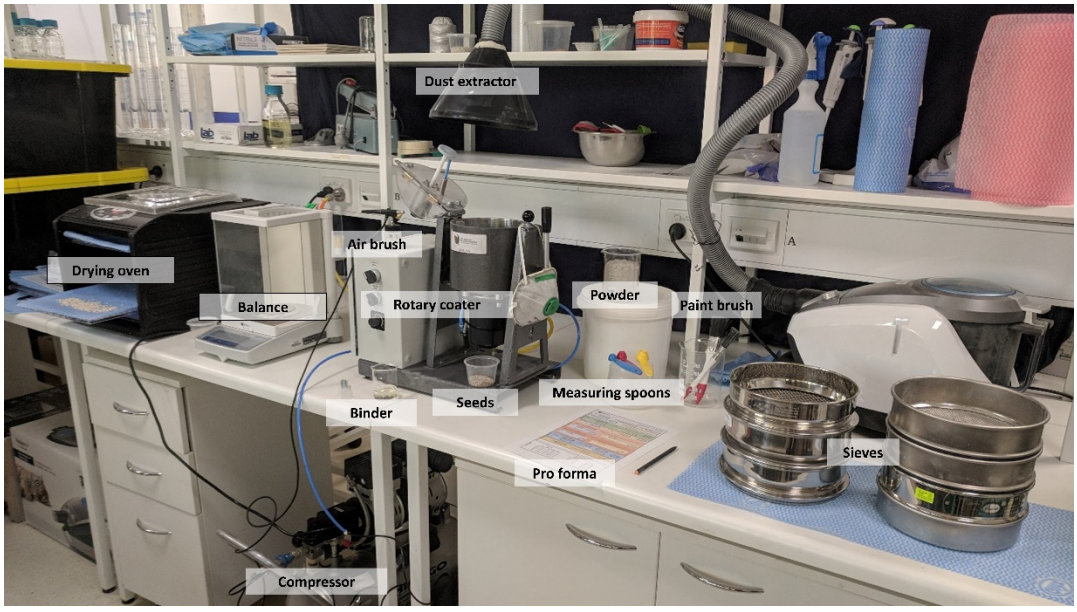



*Figure 2.2: Laboratory setting, equipment and tools required for the development of seed pelleting or encrusting protocols.*

At this stage, the air brush allows for more precise and controlled delivery of small quantities of binder compared with the more commonly used atomiser. However for larger seeds, or bigger batches of seeds, an atomiser is more appropriate (Stages 2 and

3). The atomiser is a small spinning disk suspended in the centre of the coating drum (stator). Droplets of liquid are delivered with a syringe or small tube on the spinning atomiser, where they are nebulised and distributed onto the seeds.

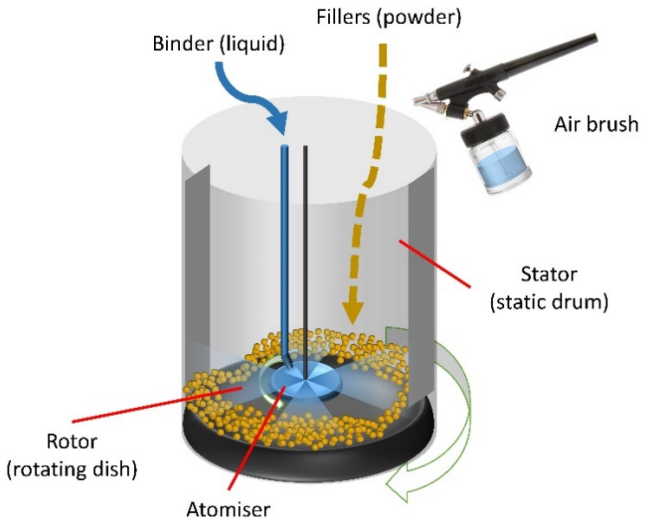



*Figure 2.3: Diagram illustrating the main components of a rotary coater. The air brush is used to apply binder in the first stage of the coating phase and it is replaced by the atomiser in stages 2 and 3. The white arrows indicate the rotation of the moving com*

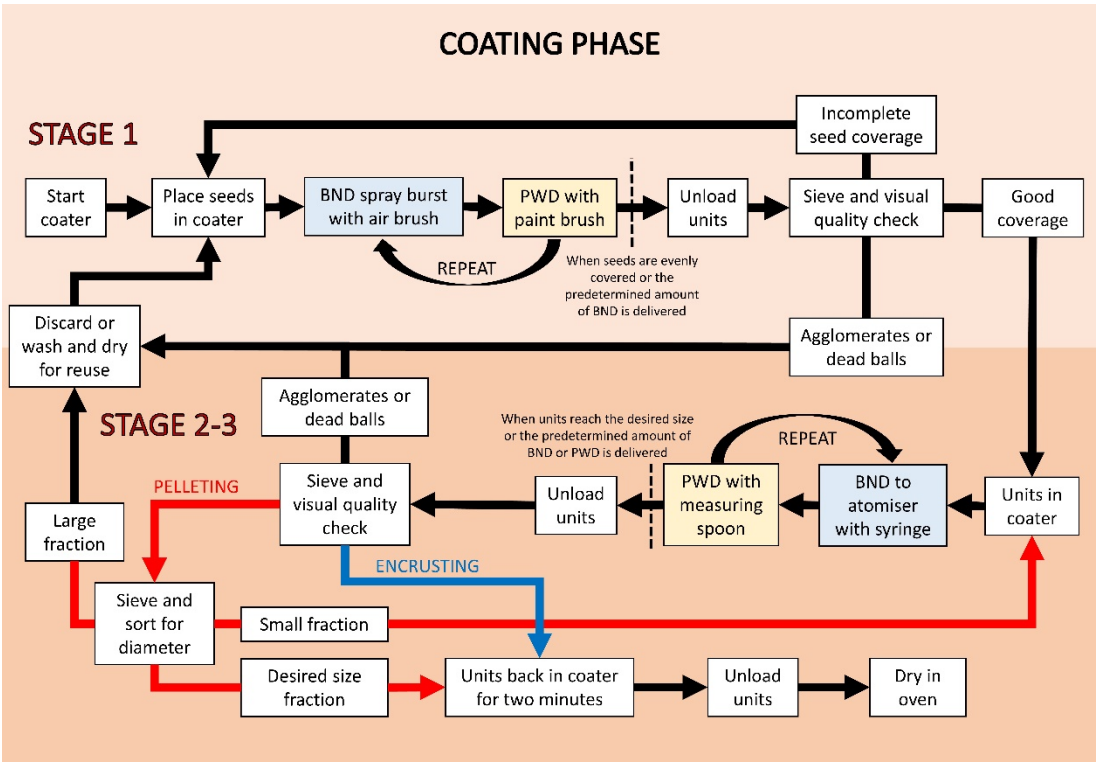



*Figure 2.4: flow chart of seed pelleting and encrusting process. The red lines indicate the pelleting, blue lines indicate encrusting, while black lines are in common for both processes. BND stands for binder and PWD for powder. Stages 2 and 3 are shown together as the process is identical, the difference being the amount of BND or PWD delivered.*

When seeds are moist, small quantities of filler are added with a paint brush that is also used to detached seeds from the wall of the stator and to stir the rotating seed mass if seeds starts to agglomerate. Binder and filler are applied alternately, in small quantities, to avoid seed agglomeration when too wet, and the formation of "dead balls" (units that contains no seeds). A small laboratory spatula is an alternative to the paint brush and may allow for more accurate powder delivery. This stage requires skill and attention and is the most important of the process as this influences the final quality of the coated seed batch. Note, if at any stage something in the process goes awry it is better to start over with a new seed batch. If seed amounts are limited or expensive (as might be the case for rare species), the failed batch can be washed free of binder and powder, dried and the process recommenced (Figure 2.3). Alternatively, for developing the protocol, use seeds of a common and inexpensive species similar in size, shape, weight, surface morphology and texture to the target species. Stage one terminates when the predetermined amount of binder has been applied and seeds are uniformly covered. Units are then removed from the coater and the quality (intactness) of the coat assessed visually. The remaining powder is weighed to determine total powder consumed in this phase.

In the next stage (Stage 2), the air brush will be replaced by the atomiser to supply binder (Figure 2.3). The binder is fed through a syringe into the pipe that delivers the material to a central rotating disk (atomiser). The liquid is nebulised upon hitting the spinning atomiser and is propelled onto the mass of rotating seeds at the periphery of the drum. It is important to adjust the height of the atomiser so that the resultant nebulised mist is applied to the centre of the rotating seed mass. To allow for faster delivery of powder, the paint brush is replaced by a measuring spoon. In order to have a correct estimate of filler delivered with each spoon, it is good practice to assess the density of the powder used. In the table (http://www.arc-cmsr.org/download/PDT_Table.xlsx), under "dosage weight", it is possible to calculate the average weight of powder contained in each measuring spoon. To do so, the spoon should be completely filled and tapped on a hard surface to allow the powder to settle; excess material is removed by levelling with a spatula and then weighed. The procedure is repeated ten times. The average weight of powder per spoonful is inserted in the "*pro forma*" and "process" page. As in the first stage, binder and filler are delivered alternately. In the examples for Stage Two, 1 g of binder is followed by a 2.5 ml (1.6 g) spoonful of diatomaceous earth. Usually during this stage one drop of binder is followed by one spoon of powder. However, this might be different with other

species/material and requires operator vigilance to constantly assess the quality of the batch and modify the quantity of powder accordingly. To keep track of these steps, every drop of binder and spoon of filler should be recorded with an "X" or a dot, on the *pro forma*. Once the desired amount of binder has been delivered, units are removed from the coater, inspected for quality, and sieved to remove smaller particles. This is the last step in the encrusting process. For pelleting, units are screened through a series of sieves of decreasing mesh size, the first being the maximum size the units should be (e.g., 4 mm), the second the minimum size (e.g., 3.15 mm) and the third a mesh size smaller than the untreated seed so as to filter out residual powder. Any unit between the minimum and maximum size should be set aside and considered complete. Larger units should be discarded and smaller units returned to the coater for the next stage.

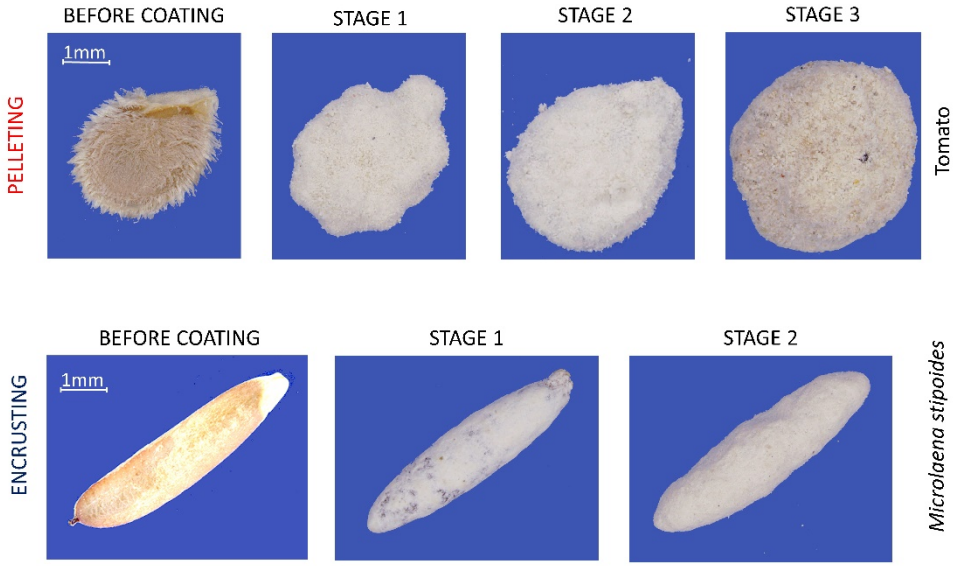



*Figure 2.5: Images of (top row) tomato seeds and (bottom row) Microlaena stipoides seeds at the end of the different stages of the coating process. At the end of stage 3 of pelleting tomato seeds, the original shape of the seeds is no longer evident, and the unit could therefore be called a pellet. Encrusting (bottom row) is a shorter process than pelleting and can be completed in two stages. It is defined as encrusting because the original shape of the seed is still evident at the end of the process.*

The following stage (Stage 3) is similar to the previous one but differs in the quantity delivered per drop, and spoon size is increased. At this stage, units are usually of sufficient size to allow for a faster build-up of material on the seeds. In the examples provided, the syringe droplet was 2 g and spoon size 5 ml (3.6 g). For each drop of binder, two levelled spoons of powder (7.2 g) were added. Once most units have reached the desired size, they should be removed from the coater and sieved for uniformity as above. Units of the desired size range should then be placed in the coater

and spun for a further two minutes at medium-high speed. This final step will drive moisture from the inner to the outer layers of the coat and would also smooth the surface (Figure 2.5).

### 2.3.4. Wet phase (WP)

Once the coating process is completed, the three size fractions (desired, small and large) should be weighed and the under and over-sized fractions discarded.

Desired size units are then placed on a tray lined with perforated cloth, and placed in a forced air drying oven or similar. In the examples provided, because these are 'developmental phase' studies, a Food Lab™ Electronic Dehydrator (Sunbeam, Sydney, Australia) was used (Figure 2.2). Any powder or binder remaining after the process should be weighed and recorded in the *pro forma* to determine the amount of raw materials used per coating batch.

### 2.3.5. Dry phase (DP)

Excessive moisture added to the units during the coating process will be removed in this phase. Temperature and duration of the drying phase is noted in the *pro forma*. In both the examples, units were dried at 35°C for three hours. Final dry weight of the desired size units is also noted in the *pro forma*. Once dried, units should be maintained at room temperature for 15-20 minutes and then stored at appropriate conditions (eg. 15% RH, 15 °C) that optimises the shelf life of coated seeds similar to untreated seeds.

### 2.3.6. Quality tests (QT)

To ensure the accuracy of pelleting or encrusting, the presence of agglomerate and "dead balls" should be checked. This is performed by crushing a minimum of five replicates of 10 units each per batch and noting the number of seeds contained in each unit (Crush test). To improve accuracy, if seed availability is not a concern, it is suggested to use 20 units per replicate in order to have a 100-unit sample size.

To ensure that units have achieved the required tensile strength to resist mechanical damage (such as during mechanised sowing), unit integrity should be checked. In the examples, an EFG1000 Digital Force Gauge (Polygon Instrument. Shenzhen, China) is used, and when the unit crumbles or exhibits cracks, the force (Newtons, N) is recorded. This test is performed on a minimum of 10 units.

In this PDT, the quality test is limited to the physical and mechanical properties of the pellet. However, additional tests for viability and germinability are strongly recommended, as one of the main features of a desired coating is that seed survival, germination and emergence are not impeded. The moisture content of pelleted and encrusted seeds is also an important parameter to take into consideration, as it may affect seed quality and physiological performance. Water activity measurement of pelleted seed can be performed following the methodology described by Taylor *et al*. (1997), allowing for optimisation of the coating and drying phase according to the species, materials and techniques used.

### 2.3.7. Summary table

Once the coating process is completed and the *pro forma* filled, the data should be inserted in the Excel file provided, in the "Process" page (http://www.arc-cmsr.org/download/PDT_Table.xlsx). This information is processed and presented in the pivot table in the "Summary" page.

The summary table is divided into nine sections. The first section describes the coating and the total time required to complete the process.

The next section, "WASTE" represents the percentage of units (in weight) that had to be discarded because of being over- or undersized (does not apply for encrusting). In the tomato pelleting example, 6.0 % of units were discarded due to either being over- or undersized. The aim is to have minimal or zero wastage.

In the "WASTE PWD" columns, the percentage of powder used that did not get incorporated but has been lost either by adhering to the inside of the coater or extracted by the dust extractor is recorded. In the pelleting and encrusting examples, the "WASTE PWD" was 65.3 and 60.9%, respectively. Although this waste could be reduced by further optimising the process, it is often difficult to have total uptake of materials onto the seeds.

The "UNITS" section provides useful information on the weight increment from original seeds to the finished units. The weight increase ratio registered for pelleted tomato seeds is 1:4.50 and for encrusted weeping grass is 1:1.57. When the same process needs to be replicated or scaled, it is crucial that the "INCREASE WEIGHT RATIO" values of the various runs falls within a small range. It should be noted that this is a

quick method of calculating weight increase and usually overestimates the actual weight increase, because it does not take into account water loss from the seeds during the final drying process. For a more accurate recording of the weight increment, it is necessary to take into account seed moisture content (Taylor 2003) both before and after the coating process, adjusting the actual weight of seeds at the end of the process to consequently correct the weight increase ratio.

The next two sections presents the results of the Quality tests (QT) as average and standard error. The results of "CRUSH TEST" for the pelleting and encrusting examples are 98 and 100% of filled units, respectively. It is important to have that value as close as 100% as possible, if the percentage of filled seeds is below 90%, it might be better to discard the batch or wash it and repeat the process.

The "COMPRESSION TEST" showed that the average force required to crack the pelleted tomato seeds was 32.94 ± 0.95 N; the force required to crack the encrusted grass seeds was 28.11 ± 2.39 N.

These first five sections of the summary table are the most useful for determining the quality of the coated batch and efficiency of the process.

The following four sections could provide important information for fine-tuning the process and optimise replicability.

The *pro forma,* and excel table provided, are a basic example of how to structure a seed coating protocol. However, for better efficiency in data collection and record management, it is worth developing a relational database, in place of the excel table, and structure the "input form" to look like the *pro forma*. This would allow for the use of hand held devices (eg. Tablets) for data entry.

## 2.4. Implications for practice

This PDT adds transparency to an industry where descriptions of products and processes has traditionally not been disclosed in a scientifically testable or publicly accessible manner. The standardised methodology presented here allows for scientific evaluation of the effect of variables such as different species and seed lots, coating materials, process duration, number of layers, and drying conditions on the quality and integrity of coated seeds, and can be used to generate readily replicated protocols.

The processes outlined here can also be used to test the efficacy of different active compounds and biological adjuvants such as soil microbes and their products as components in the coating process.

As the PDT employs standard equipment, the processes allow for up-scaling and can be easily adapted to industrial-scale operations. The processes outlined provide significant commercial and seed-use efficiencies for small companies and organisations where out-sourcing seed coating is costly or where there is a lack of 'know-how' to effectively apply reliable and consistent seed treatments. This is particularly true for the burgeoning native seed industry, an industry predominantly comprising small- to medium-sized enterprises dealing with many (perhaps hundreds or thousands) different species (De Vitis *et al*., 2017) yet never or rarely deploying encrusted or pelleted seeds (Pedrini *et al*., 2017). This PDT provides a standardised protocol for coating that can be tested on and applied to a diverse range of native seeds, allowing for the implementation of coating in the native seed production and processing chain. Seed-based ecological restoration is emerging as a major global industry that could significantly benefit from the application of advanced seed coating technologies. Given the need to deliver multispecies seed mixes at large scales, often to challenging substrates and environmental conditions (e.g., the restoration of post-mining landforms), there is an urgent need for practical and scientifically-tested seed encrusting and pelleting solutions to improve seed-use efficiency, the delivery of seeds to site and the cost-effectiveness of landscape-scale restoration.

## 2.5. Bibliography

De Vitis M, Abbandonato H, Dixon K, et al (2017) The European Native Seed Industry: Characterization and Perspectives in Grassland Restoration. Sustainability 9:1682 . doi: 10.3390/su9101682

Halmer P (2000) Commercial seed treatment technology. In: I. Black M, Bewley JD (eds) Seed Technology and its biological basis. Sheffield Academic Press, Sheffield, pp 257–283

Hill HJ (1999) Recent Developments in Seed Technology. J New Seeds 1:105–112 . doi: 10.1300/J153v01n01_09

Howard PH (2009) Visualizing consolidation in the global seed industry: 1996-2008. Sustainability 1:1266–1287 . doi: 10.3390/su1041266

Kaufman G (1991) Seed Coating: A tool for stand establishment; a stimulus to seed quality. Horttechnology 1:98–102

MarketsandMarkets (2016) Seed Coating Materials Market by Type (polymers, Colorants, pellets, minerals/pumice, and Other Additives), Crop Type (Cereals & Grains, Oilseeds & pulses, Fruits & Vegetables, flowers & ornamentals), & by Region - Global Trends & Forecasts to 2020

Mathre DE, Johnston RH, Grey WE (2001) Small Grain Cereal Seed Treatment. Plant Heal Instr. doi: 10.1094/PHI-I-2001-1008-01

Menz MHM, Dixon KW, Hobbs RJ (2013) Hurdles and Opportunities for Landscape-Scale Restoration. Science (80- ) 339:526–527 . doi: 10.1126/science.1228334

Merritt DJ, Dixon KW (2011) Restoration Seed Banks--A Matter of Scale. Science (80- ) 332:424–425 . doi: 10.1126/science.1203083

Pedrini S, Merritt DJ, Stevens J, Dixon K (2017) Seed Coating: Science or Marketing Spin? Trends Plant Sci 22:106–116 . doi: 10.1016/j.tplants.2016.11.002

Scott JM (1989) Seed coatings and treatments and their effects on plant establishment. Adv Agron 42:43–83

Taylor AG (2003) Seed Treatments. In: Seed development. pp 1291–1298

Taylor AG, Allen PS, Bennett M a., et al (1998) Seed enhancements. Seed Sci Res 8:245–256 . doi: 10.1017/S0960258500004141

Taylor AG, Grabe DF, Paine D. (1997) Moisture content and water activity determination of pelleted and film-coated seeds. Seed Technol 19:24–32

Taylor AG, Harman GE (1990) Concepts and Technologies of Selected Seed Treatments. Annu. Rev. Phytopathol. 28:321–339

# 3. Seed pelleting: trade-off between germination and mechanical integrity

## 3.1. Introduction

Seed coating treatments are a standard procedure in the crop and vegetable seed industry. Their deployment, already common in western markets, is rapidly expanding into the developing world. According to recent market estimates, the value of seed coating material alone is expected to reach $2.21 billion globally by 2023 (Stratistics 2017).

The widespread use of seed coating technology is explained by its efficiency in carrying and delivering specific compounds that could either protect the seed from disease or predation (Almeida et al. 2010; Song et al. 2014), enhance seed germination (Wang 2012), seedling survival (Murata et al. 2008) and yield (Gevrek et al. 2012). Seed coating is also used to modify seed shape, size, weight and density (Taylor and Harman 1990). These physical modifications allow for seeds of varying morphologies to be of standardised unit size leading to improvement in the efficiency and consistency of seed flow through seeding devices such as air seeders and seed drills. (Scott 1989). Seed coatings can be categorised according to the degree to which the size of the seed is increased. Seed pelleting is the type of seed coating that involves the greatest modification of size. A pelleted seed is covered by layers of coating material to the degree that the original shape of the seed is no longer evident, and the unit is roughly spherical (Taylor et al. 1998). The materials that constitute a pellet could be broadly categorised into two groups: *binders* usually in liquid form, that provide adherence to the seed and are responsible for the mechanical integrity of the coating; *fillers*, usually delivered in powder form, are bulking agents responsible for the increase in weight and size (Pedrini et al. 2018a).

### 3.1.1. *Trade protection of protocols and materials*

The commercial seed sector has developed hundreds of products for seed coating and pelleting, and many protocols for specific seed types that are crop and industry specific. However, most of this technological development is not publicly accessible, nor replicable due to trade protection (Halmer 2008). Indeed, a recent review (Pedrini et al. 2017) highlighted that the coating process was outsourced to private companies

and, therefore, not disclosed in half of the scientific publication available in the public domain. Recent studies have tried to make seed coating and pelleting information more accessible to scientists and seed producers, particularly to those who cannot access the highly protected industry trade secrets (Pedrini et al. 2018a). When specific studies have been performed to evaluate the mechanical properties of pelleted seed, proprietary products of unknown composition were used (Taylor et al. 1997; Grellier et al. 1999). This emphasises the need for a systematic evaluation of fully disclosed seed pelleting materials, to improve understanding of the mechanical behaviour of pellets and how pelleting materials affect germination.

### 3.1.2. *Seed pelleting effect on germination*

Seed germination response to pelleting treatment is not consistent through the literature. Improved germination performance of pelleted seeds, compared to untreated seeds, have been reported for lettuce (*Lactuca sativa)* (Zuffo et al. 2017), and guayule (*Parthenium argentatum)* (Sanchez et al. 2014). However, delays and reduction in seed germination, or seedling emergence, were reported for sweet corn (*Zea mays*) (Somrat et al. 2017), sugar beet (*Beta vulgaris*) (Arshadi and Asgharipour 2011), tomato (*Solanum lycopersicum*) (Govinden-Soulange and Levantard 2008), white clover (*Trifolium repens*) (Esfahani and Shariati 2006), bean (*Phaseolus vulgaris*) and mung bean (*Vigna radiata*) (Babu et al. 2005). Due to the great variability in response of different species to pelleting treatment, and patchy information concerning pelleting material and methods, it has so far been difficult to predict the germination response of pelleted seeds and understand the mechanism underlying such responses. Sachs et al. (1981) suggested that pelleting could affect oxygen availability to the embryo, altering germination behaviour. Water retention proprieties (Grellier et al. 1999), and moisture content and water activity (Taylor et al. 1997) of pelleting materials (fillers), in relation to the potential limitation in oxygen availability, were described but were not tested against germination performance. In some cases, authors suggested that the layers of pelleting material might have provided physical constraints to the emerging radicle (Govinden-Soulange and Levantard 2008).

An important property of pelleted seeds is mechanical integrity. A pellet should have sufficient structural integrity to survive drying, packaging, distribution, storage and deployment, without breaking or crumbling (Hill 1999). Mechanical integrity and the ability to retain active ingredients onto the seed is paramount (Nuyttens et al. 2013),

especially when pellets are loaded with compounds, such as pesticide, that can be harmful to the human operators and the environment. This has been a priority in industry research and development, but little work has so far been undertaken within the scientific community in addressing pellet integrity and retention of active ingredients. The mechanical integrity of a pellet is a function of its components (Somrat et al. 2017), especially binder concentration and binder interaction with different powders (Hill 1999). Thus, we hypothesised that increased binder concentration can, on the one hand, improve pellet resistance to mechanical stress, but on the other hand, be responsible for impeding seed germination. Whether those limitations are due to mechanical resistance from the pelleting material, or due to modification in water availability to the seed, is unclear.

The aim of this study was to understand the effect of seed coating materials on germination based on the key questions:

- Does the seed pelleting process reduce seed germination?
- What are the physical properties of pellets, comprising different constituent powders and binder concentration, in terms of resistance to mechanical stress and imbibition of water?
- Does seed pelleting with different fillers and binder concentration affect seed germination at full and/or reduced water potentials? If so, is this effect correlated with the physical proprieties of the pellets?

If correlations between seed germination and physical proprieties of the pellets are detected, the ultimate aim of this study was to determine, for each of the fillers tested, the optimal binder concentration that would allow for the best mechanical proprieties, with the least detrimental effect on germination outcomes.

## 3.2. Material and methods

### 3.2.1. *Species*

Raw (untreated) tomato seed (*Solanum lycopersicum* L.), variety Tytanium, were donated by HM Clause Vegetables and stored in sealed foiled bags at ambient temperature 20-25° C.

### 3.2.2. *Seed pelleting*

#### *3.2.2.1. Binders*

Of the binders most commonly reported in the literature, hydroxyethyl cellulose (HEC) was chosen for experiments because it provides comprehensive seed coverage, is strongly retained on the seed, whilst being chemically neutral and, thus, not imparting effects on seed germination (Almeida et al. 2005). HEC is a non-ionic, water-soluble polymer derived from cellulose. It is commonly used as binding, thickening, stabilizing and film forming agent, is easily soluble both in cold and hot water, and it is available in a variety of viscosity grades (Abdel-Halim 2014). Of the commercially available grades, CELLOSIZE QP 09-L (DOW chemicals) was chosen for its lower viscosity range of aqueous solution that would allow for spray delivery (Dow 2005). HEC solutions were prepared by dusting CELLOSIZE QP 09-L on 100ml of deionised water at 20° ± 2° and mixing on a magnetic stirrer for 10 minutes. HEC was applied at 1%, 2 %, 3% and 4% w/v solution. Higher concentrations could not be tested, as the viscosity for higher concentrations of HEC solution renders the delivery through an airbrush unfeasible.

#### *3.2.2.2. Fillers*

Diatomaceous earth (DE) and talc (TL) were selected to be used as filler. Both materials have commonly been reported in the literature and used in the industry for seed pelleting and encrusting (Taylor and Harman 1990; Pedrini et al. 2017). DE is powdered fossilised microscopic algae (diatoms). It is a low density, high porosity material, that is used in seed pelleting as a bulking agent and for its insecticidal proprieties (Fields et al. 2002). Talc is commonly used for seed coating because of its propriety of reducing friction (Nuyttens et al. 2013) improving seed flow, and enhancing the aesthetic appearance of pelleted seed (Halmer 2008).

#### *3.2.2.3. Process*

Seed pelleting was performed on an RRC 150 Lab Coater (Centor Thai, Bangkok, Thailand). Each pelleting treatment was performed on an 8 g batch of seed, following the protocol development tool developed by Pedrini et al. (2018). The pelleting process was divided into three stages. In the first stage HEC solution, or water, was delivered via an Air Compressor propelled 0.8 mm Air Brush (Voliamart, Reservoir, Australia) kept at a distance of 10-15 cm from the rotating seed mass. The first step of the process

was the application of liquid, to increase seed moisture and allow for powder retention. The powder was added gradually using a brush. Liquid and powder were added alternately. During the initial liquid-powder cycles, limited quantities were applied to avoid seed agglomeration when too wet and the formation of empty pellets (that contains no seed). As the pellets increased in size and weight, materials were added in larger quantities. When 10 ml of binder were used, seeds were removed from the coater and sieved, to remove the excess powder, and the quality of the batch visually assessed. In the second stage, the liquid was delivered through the spinning disk that sits in the middle of the coating drum (atomizer). Pellet quality was visually inspected during the process, on randomly selected samples. In the final stages, when most of the pellets had reached the desired minimum size (3mm in diameter), they were removed from the coater and passed through a 3 mm sieve. Pellets smaller than 3 mm were returned in the coater and processed until reaching 3 mm diameter. The duration of the process was recorded. At the end of the process, pelleted seeds were dried at 35° C for 3 hours in a Food Lab™ Electronic Dehydrator (Sunbeam, Sydney, Australia). After drying, five subsamples of 10 pellets were collected from each batch to check for dead balls or doubles. After drying, the pellets were sorted through 3 and 4 mm sieves. Smaller and larger pellets were separated and not used in the experiments because high variability in pellet size could have biased seed germination results.

### 3.2.3. *Physical proprieties of pelleted seeds*

#### *3.2.3.1. Mechanical stress resistance*

The structural integrity of pellets was assessed by testing the resistance to mechanical stress, following the compression test method described by Pedrini et al. (2018). The test was performed on five replicates of ten pellets randomly selected for each treatment. Each pellet was placed on a flat and smooth surface and subjected to gradual mechanical compression with an EFG1000 Digital Force Gauge (Polygon Instrument, Shenzhen, China). The gradually increasing pressure was applied until the pellet had crumbled or cracked, and the force (N) required to crack the pellet was recorded.

#### *3.2.3.2. Water imbibition*

Water imbibition curves were obtained by measuring relative seed moisture content at different imbibition times. 60 pellets per treatment were placed on a 90 mm Petri dish

lined with two filter papers. 14 ml of deionised water was added. After 30 seconds, free-flowing water on the surface of the filter paper was removed with a syringe. Petri dishes were sealed with glad wrap and left at ambient condition (20-25°C) at predetermined imbibition times (30 minutes, 1, 2, 4, 8 and 24 hours). Pellets were then removed from the petri dish, placed in a fine mesh sieve, crushed with a spoon and submerged in water for one second. This operation was repeated three times to allow for the complete removal of the pelleting material. Unpelleted control seeds were also subjected to this operation.

The cleaned seeds were placed on a dry filter paper, and excess water still present on the seeds' surface after the cleaning process was removed by tapping a dry filter paper on top of the seeds for five seconds.

Five replicates of ten seeds were then weighed. Seeds of each replicate were then placed in paper bags and oven dried at 103 °C for 17 hours (ISTA 2007). After drying, the seeds were left cooling at room temperature (20-25°C) for 30 minutes and then weighed again. Relative seed moisture content was calculated with the following equation:

$$\left(\frac{Wet\ seed\ weight - Dry\ seed\ weight}{Dry\ seed\ weight}\right)$$

### 3.2.4. *Germination experiments*

Seed germination was tested in 90 mm Petri dishes, on two sheets of filter paper moistened with 14 ml of water. To avoid desiccation, Petri dishes were placed in Ziplock bags, and 2 ml of water or solution were added weekly. 50 seed were placed in each petri dish with five replicates per treatment.

Sown petri dishes were incubated at 25°C in Biosyn 6000 OP (Contherm, Korokoro, New Zealand) with an alternating light/dark photoperiod of 12 hours. Germination was recorded when radicle protrusion was detected. To account for the extra distance required for the radicle to emerge through the layer of pelleting material, germination was recorded on untreated seed at 0.5 mm length, whilst at 2 mm length for pellets. To

simulate reduced water availability, filter papers were imbibed with a polyethylene glycol PEG 6000 (Sigma Aldrich, St. Louis, USA) solution of 202.13 g/l adjusted to 25°C (Michel and Kaufmann 1973) resulting in water potential of -0.5 Mpa, tested with WP4C Dewpoint PotentiaMeter psychrometer (Decagon Devices, Pullman, USA). Germinated seeds were removed from the petri dish. Germination was scored every 12 hours for 7 days at full water availability and daily for 17 days at reduced water availability. On seed and pellets that had not germinated a cut test was performed to assess seed viability and fill (for pellets). Non-viable seeds and empty pellets were not considered for the data analysis.

#### *3.2.4.1. Pelleting process effect on germination*

To determine if the seed pelleting process had a detrimental effect on germination performance, untreated control (CTRL) seed were compared with seeds that had been pelleted but had the external pelleting material crushed and removed (CRSH). 0.5 g of pellets were randomly selected from each of the ten pelleting treatments, and then cleaned of the coating materials following the procedure previously described for the water imbibition test. Germination was tested at the conditions reported in the "germination experiments" section.

#### *3.2.4.2. Pelleting material effect on germination*

Germination was tested in a controlled laboratory environment at an optimal temperature condition of 25°. Two separate experiments were performed to assess germination at full water availability and reduced water availability of -0.5 Mpa, using a PEG solution, following the procedures described above. A total of 10 pelleting treatment resulting from the combination of five binder (HEC) concentrations (0%, 1%, 2%, 3%, and 4%), and two powders (talc and diatomaceous earth) were tested.

### 3.2.5. *Data analysis*

To determine cumulative seed germination and water imbibition over time, for the various seed pelleting treatment, data analysis was performed using the software R (R Core Team 2015). Non-linear regression models were fitted with the function "drm" of the "DRC" package (Ritz et al. 2005, 2015). A three-parameter log-logistic model was used:

$$f(x) = \frac{gmax}{1 + \left(\frac{x}{T50}\right)^b}$$

The parameters are: (b) slope curvature, (gmax) final germination or final imbibition and (T50) germination speed or imbibition rate, intended as time (hours or days) required to reach half of the final germination/imbibition. Parameter comparison on final germination and germination speed, and final imbibition and imbibition rate, were then performed to assess differences among treatment (significance $p < 0.05$).

Correlation between variables was performed with the "cor()" function in R using the "pearson" methods. The correlation was considered significant ($p < 0.05$) when correlation coefficient (R) was higher than the 95 Percent Critical Value of the Sample Correlation Coefficient table (Texasgateway.org 2007) for the appropriate degree of freedom (3 degrees of freedom, value = 0.878). For a visual representation, linear regression of the averages for the variables were obtained with the "lm()" function in R.

To perform the trade-off analysis between germination performances (Final germination and T50) and mechanical stress resistance, all dataset were transformed to fit within a 0 to 1 range, using the following formula:

$$z_i = \frac{x_i - \min(x)}{\max(x) - \min(x)}$$

For germination speed (T50), normalised data were transformed further by inverting max and min ($1\text{-}z_i$). Transformed data were then fitted in linear regression for each powder, and water potential combination separately. Regression lines for germination response (final or T50) and mechanical stress resistance were plotted and the intersection between the lines calculated using the "linear model intercept function" described by Matt L. (stackoverflow.com 2016). The "x" value of the intersection represents the optimal binder concentration resulting from the trade-off between germination performances and mechanical stress resistance. The "y" value of the intersection was then converted back to the original variable scale in day or hours for T50 and in N for mechanical stress resistance.

## 3.3. Results and discussion

### 3.3.1. *Seed pelleting process effect on germination*

Final germination of seeds that have been pelleted and then completely cleaned of the coating material was 97.9 ± 0.7%, which is slightly lower, but not significantly different ($P < 0.05$) to the final germination of untreated seeds (98.9 ± 0.7%). Similarly, there was no significant difference in germination speed (T50), with cleaned seed geminating slightly faster (41.9 ± 0.8 hours) than the untreated control (42.2 ± 0.5 hours). These results suggest that the pelleting process, applied in this study, is not influencing germination outcomes, leaving the physical proprieties of the materials as the main factor affecting seed germination.

### 3.3.2. *Physical proprieties of pelleted seeds*

#### *3.3.2.1. General pellet proprieties*

All pelleting treatments were performed with the goal of obtaining homogeneous pellets of a size range between 3-4 mm in diameter. However pelleting processes requires some degree of craft and experience (Halmer 2000) by the operator to address potential issues (e.g. formation of empty pellets, agglomeration, coating material deposition in the coater) arising during the process. These adjustments had some impacts on process duration (min 30, max 55 minutes), relative weight increase, and pellet quality (Table 3.1). However, these variabilities were within a reasonable range, with standard error relative to the average, lower than 5%.

The pellets made with talc were 99.6% filled, with the only defect detected at the 3% binder concentration, where 2% of the pellets were empty. Quality of DE pellets was lower with an average of 95.6% of pellets filled and 2.4% empty and 2% agglomerates. Relative weight increase for DE pellets averaged 5.33 ± 0.10, and for TL 8.75 ± 0.14. This difference in relative weight increase is due to the higher density of TL over DE.

Density is an important characteristic of pelleted seed in relation to its intended use. Historically, lighter pellets have been preferred for glasshouse production, while heavier pellets are used for in-field sowing (Hill 1999).

*Table 3.1: Seed pelleting process specifications for the ten pelleting treatments. The first two columns show the 10 treatments based on various powders (PWD): talc and diatomaceous earth (DE), and five binders (BND) concentrations. The binder used was hydroxyl ethylcellulose. Specification of the different treatment, such as pelleting process duration, initial seed weight, final pellet weight and weight increase proportion are reported. The last three columns form part of the seed pellet quality test, were a subsample of 50 pellets from each treatment were crushed and examined to assess the percentage of pellets with a seed (filled), pellets that contain no seed (empty) and pellets that contain more than a seed (agglomerate).*

| PWD | BND conc. | Process duration (min) | Initial seed weight (g) | Final pellet weight (g) | Weight increase | Filled pellet | Empty pellets | Agglomerate pellets |
|---|---|---|---|---|---|---|---|---|
| DE | 0% | 30:00 | 8.12 | 40.21 | 4.95 | 100% | 0 | 0 |
| | 1% | 43:00 | 8.03 | 43.16 | 5.38 | 98% | 2% | 0 |
| | 2% | 44:00 | 8.03 | 45.13 | 5.62 | 98% | 2% | 0 |
| | 3% | 34:00 | 8.03 | 42.20 | 5.26 | 98% | 2% | 0 |
| | 4% | 43:00 | 8.03 | 43.73 | 5.44 | 84% | 6% | 10% |
| | **Avg.** | **38:48** | **8.05** | **42.88** | **5.33** | **95.6%** | **2.4%** | **2%** |
| TALC | 0% | 45:00 | 8.01 | 72.60 | 9.06 | 100% | 0 | 0 |
| | 1% | 37:00 | 8.02 | 69.24 | 8.63 | 100% | 0 | 0 |
| | 2% | 55:00 | 8.02 | 66.05 | 8.24 | 100% | 0 | 0 |
| | 3% | 38:00 | 8.01 | 70.31 | 8.78 | 98% | 2% | 0 |
| | 4% | 43:00 | 8.01 | 72.64 | 9.06 | 100% | 0 | 0 |
| | **Avg.** | **41:12** | **8.02** | **70.17** | **8.75** | **99.6%** | **0.4%** | **0%** |

#### *3.3.2.2. Resistance to mechanical stress*

The increase in binder concentration had a significant effect on improving resistance to mechanical stress of pelleted seed (Figure 3.1), but the effect varied according to the powder used. With talc and water as a binding agent (BND 0%), there was insufficient mechanical resistance (Force < 0.1 N). The pellets resulting from this treatment had to be handled with extreme care for the germination and imbibition experiment, to avoid breakage and crumbling that could have biased the results. As binder concentration was increased, resistance to mechanical stress was also improved until reaching 20.6 ± 0.9 N at 4% HEC concentration (Figure 3.1).

For pellets made of diatomaceous earth, water alone provided some mechanical integrity and resistance to stress (5.2 ± 0.7 N). At 1% binder concentration, DE pellets were still more resistant than TL pellets. At 2 and 3%, mechanical resistance was quite similar, while at 4% TL was slightly higher than DE. For both powders, the correlation between mechanical stress resistance and binder concentration was significant (Figure 3.1).

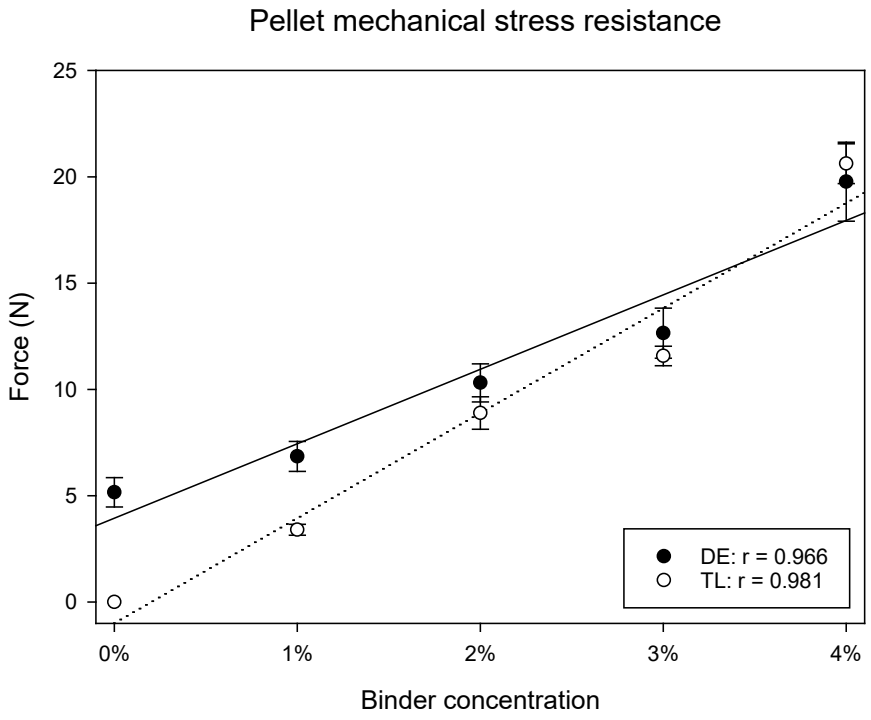



*Figure 3.1: Correlation between pellet mechanical resistance to stress (N) and concentration of the binder hydroxyl ethylcellulose (HEC). Open dots, with a dotted regression line, represent the pellets prepared with talc (TL). The dark dots and solid line are for diatomaceous earth (DE) pellets. The correlation coefficient (r), shown in the legend, is considered significant when higher than 0.875 (95 Percent Critical Value with a degree of freedom of 3)*

These results are consistent with that shown by Somrat et al. (2017), where increased concentration in two of the three tested binders (gelatin and gum arabic) improved mechanical integrity of the pellet on sweet corn.

#### *3.3.2.3. Water imbibition*

When tested at full water availability, the average weight increment for all treatment after 24 hours was 48 ± 1.6%, and the time required to reach 50% of imbibition (T50) was on average 34:46 ± 3:31. None of the treatments tested had any significant difference to the untreated control, for final imbibition and imbibition rate (T50).

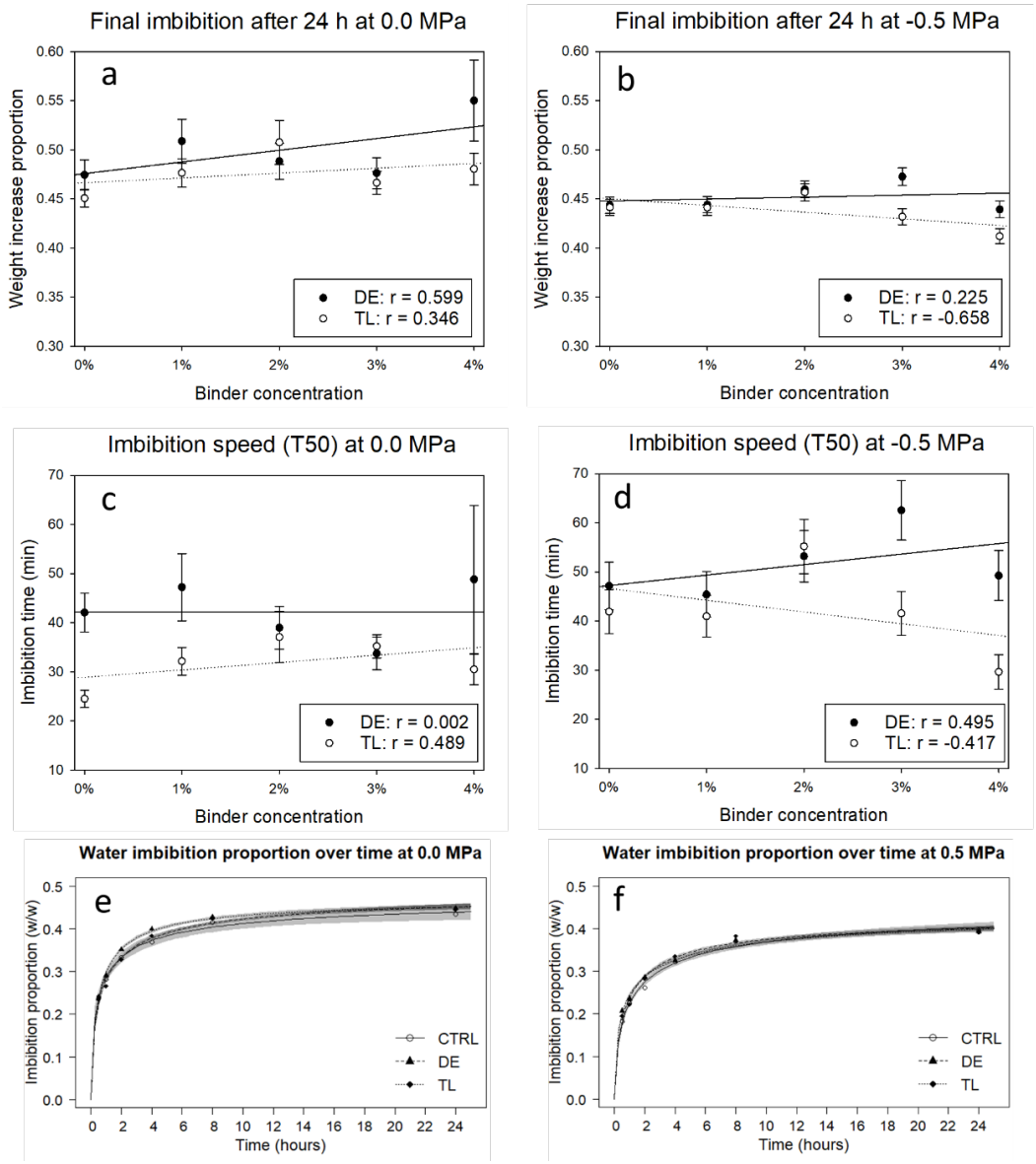



*Figure 3.2: Weight increase of pelleted seed imbibed at two different water potentials (0.0 and -0.5 MPa). Graphs "a" and "b" present the correlation between weight increase proportion and concentration of polymer (HEC). Graphs "c" and "d" present the time (min) required to reach half of the final water absorption. The open dots, with a dashed regression line, represent the pellets prepared with talc (TL). Solid dots and solid line are for diatomaceous earth (DE) pellets. The correlation coefficients (r), shown in the legends, are considered significant when higher than 0.875 (95 Percent Critical Value with a degree of freedom of 3). "e" and "f" present the cumulative imbibition curve of untreated seed (CTRL) and pelleted with talc (TL) and diatomaceous earth (DE) at 0.0 and -0.5 MPa. Results were estimated using a three-parameter log-logistic curve. The lines represent the cumulative imbibition curve (proportion) over time. The dots indicate imbibition recorded on a specific hour and shaded areas around the germination curves are the 95% confidence intervals.*

Comparing binder concentrations, 0% had the lowest final moisture content (significantly lower than 2% and 4%, $P<0.05$), but the fastest imbibition rate, (significantly faster than 1% and 2%, $P>0.05$). DE had the highest final water imbibition, although not significantly different from TL and the control, but the imbibition speed (T50) was 10 minutes slower than for TL ($P<0.001$) (Figure 3.2).

When water availability was limited by using a PEG solution set at -0.5 MPa water potential, the average weight increment after 24 hours was 44 ± 1.6%, and T50 was reached at 49:20 ± 6:07 min, both lower and slower than when imbibition was tested in water. There was no significant difference in final imbibition between binder concentrations ($P < 0.05$). However, the untreated seed had a slower imbibition rate, with T50 reached at 64:50 ± 6:06 min, significantly different from 0, 1 and 4% HEC concentration ($P<0.05$). For each powder at different water potential the correlations between final imbibition or imbibition rate (T50) and binder concentration were not significant ($P > 0.05$).

Differently to the resistance to mechanical stress, where a clear correlation with binder concentration was found, the capability of seed of imbibing water through layers of different pelleting material and at different binder concentration was not detected. If different response in germination are found among different treatment, the most likely cause for the effect would be mechanical resistance, not water absorption proprieties of pelleting materials. In the following steps, when germination outcomes (final and T50) are correlated with physical proprieties of the pellets, imbibition results will not be taken into consideration.

### 3.3.3. *Seed pelleting material effect on germination*

Final germination for untreated tomato seed was 98 ± 0.7% and had reached T50 in 42.1 ± 0.5 hours. When germination was tested at low water availability (-0.5 MPa), final germination of untreated seed was 93 ± 1.2% and reached T50 after 4.88 days.

#### *3.3.3.1. Powder effect*

When powder effects on germination were compared, without taking into consideration binder concentration, DE reduced final germination by 2.37% compared to TL. A similar limitation in DE was detected at -0.5 MPa but the difference was not significant. In both water availability scenarios, both powders had significantly ($P < 0.05$) lower final germination compared to the control. Between powders, germination speed was not affected at 0.0 MPa, but with reduced water availability, germination speed was 0.4 days slower in seed pelleted with DE, compared with TL. As for final germination, T50 was significantly faster ($P < 0.05$) in the unpelleted seed.

#### *3.3.3.2. Binder concentration effect*

Compared to the control, all seed pelleted with DE, at all binder concentrations, showed a significant drop in final germination between 4 and 5% ($P < 0.001$). However, there was no difference between any of the concertation tested. These limitations in germination for DE pelleted seeds might be due to physical properties of the material that have been known to reduce oxygen availability (Sooter and Millier 1978).

For TL pelleted seed, lower binder concentration (0 and 1%) provided no difference in germination compared to the control, whilst the 2, 3 and 4% concentration reduced final germination, however, when compared with each other, there was no significant difference among binder concentration. The effects of pelleting on final germination were less evident at -0.5 MPa than at 0.0 MPa, with DE at 0 and 2%, and TL at 0, 1 and 3% not significantly different to the control ($P < 0.05$). No difference was detected among binder concentration for DE, while 4% concentration had a significant detrimental effect compared to 0, 1, and 3%. At 0.0 MPa in both powders, germination speed (T50) was similar to the control when water was used as a binder, but slower when HEC was used at any concentration (Figure 3.3). In the -0.5 MPa germination experiment, T50 was significantly slower for all treatment tested with the exception for TL at 0%. Similar to the 0.0 MPa scenario, there were significant differences between binder concentrations with higher binder concentrations usually resulting in slower germination (Figure 3.3).

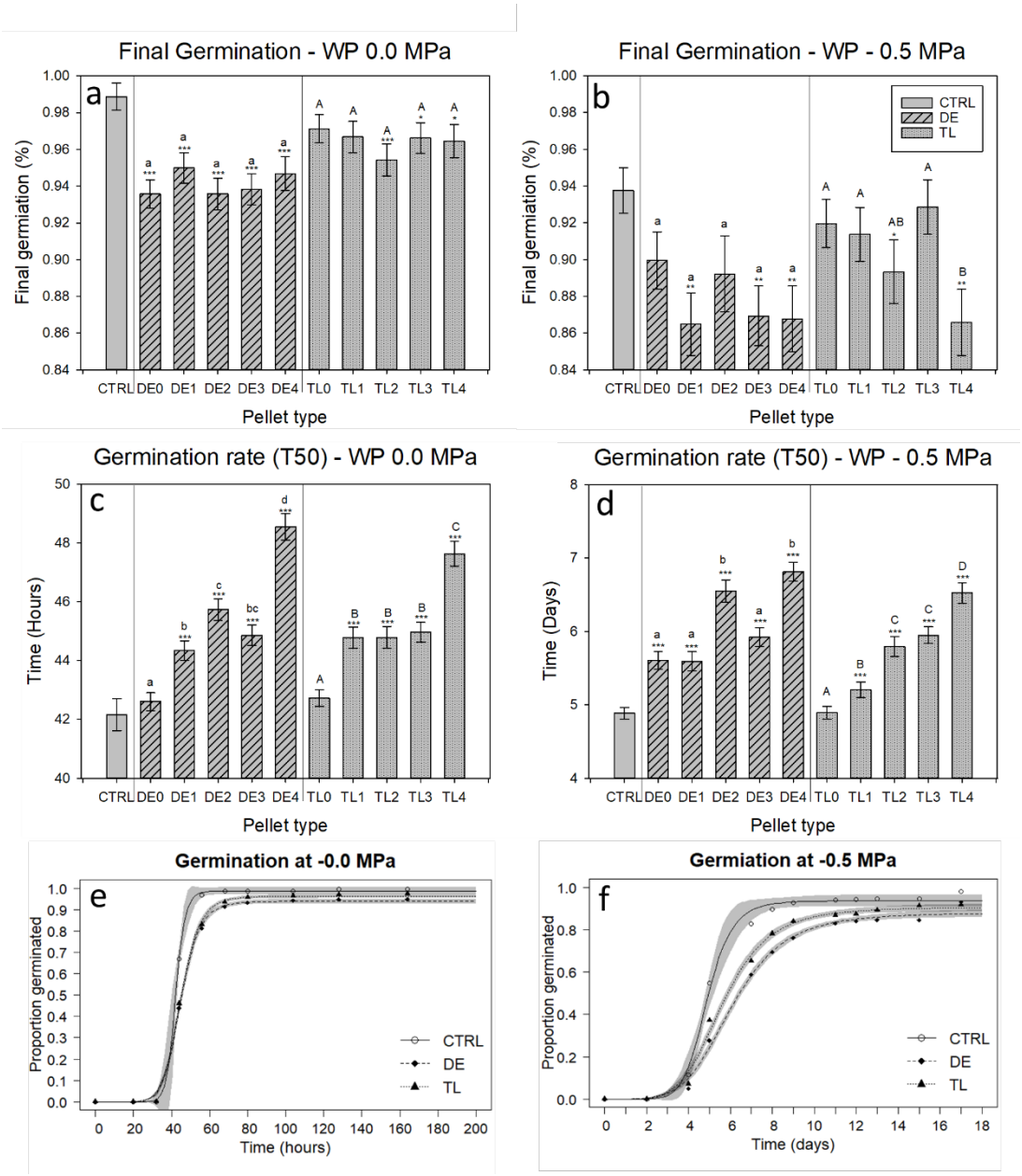



*Figure 3.3: Seed germination at two different water potentials: 0.0 MPa on the left and -0.5 MPa on the right. The histograms "a" and "b" present the final germination of the control (CTRL) and each pellet treatment with diatomaceous earth (DE) and talc (TL) at five different polymer concentration (0-4%). The histograms "c" and "d" present the germination speed in Hours (for 0.00 MPa experiment) and in days (for the -0.5 MPa experiment). Significance between the control and the pelleting treatments is marked with the * sign (* P<0.05, **P<0.01, ***P<0.001). Significance between binder concentrations for each powder are represented where values with same the letter are not significant, with lower case for DE and capital letters for TL. Results followed by the same letter are not statistically different at p < 0.05. The graphs "e" and "f" represent cumulative germination for untreated seed (CTRL) and pelleted with talc (TL) and diatomaceous earth (DE) at 0.0 and -0.5 MPa. Results were estimated using a three-parameter log-logistic curve. The lines represent the cumulative imbibition curve (proportion) over time. Imbibition is recorded hourly. Shaded areas around the germination curves are the 95% confidence intervals.*

*3.3.3.3. Correlation between germination performances and pellet physical proprieties*

Germination outcomes for the various pelleting treatment were plotted against the mechanical resistance value of each specific treatment, to detect potential correlations. These correlations were not performed for water imbibition, because previous results showed no correlation between increased binder concentration and imbibition rate.

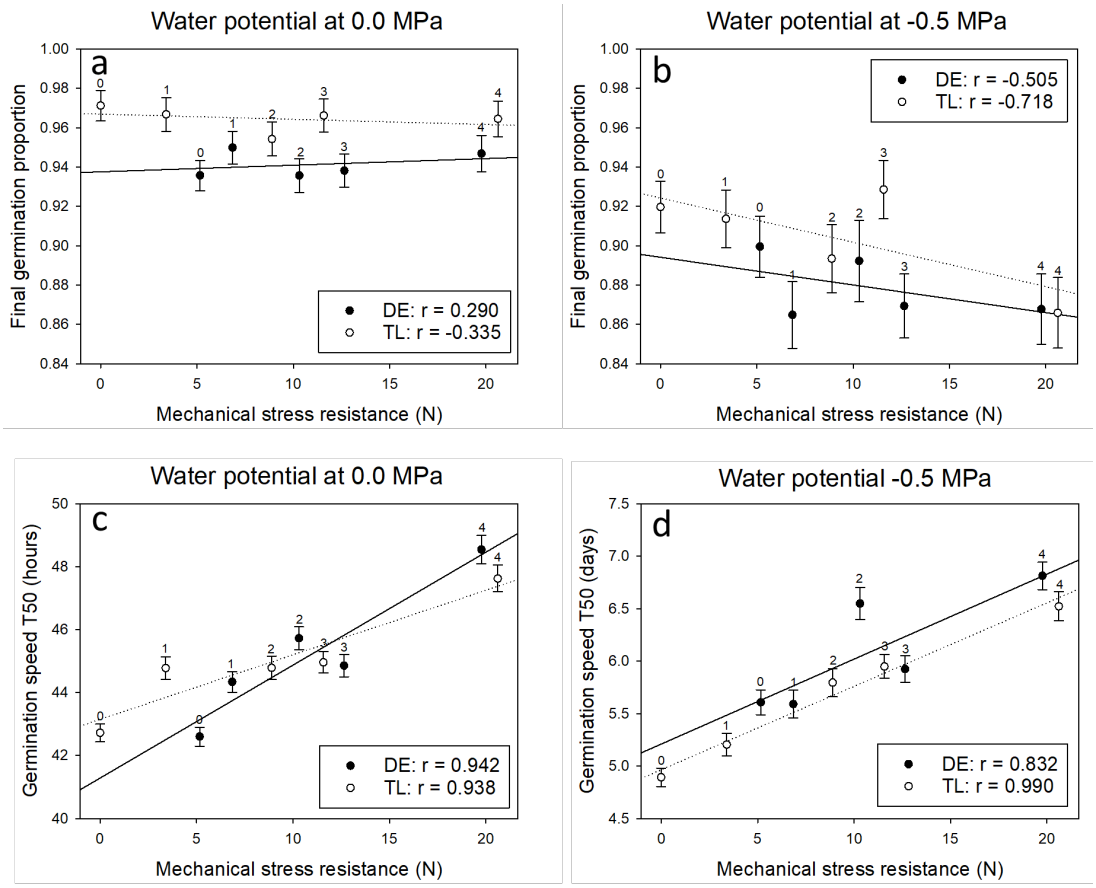



*Figure 3.4: Correlations between germination performance of pelleted seed and mechanical stress resistance. The graphs "a" and "b" present final germination at 0.0 and -0.5 MPa and graphs "c" and "d" germination speed (T50). Open dots, with a dotted regression line, represent the pellets prepared with Talc (TL). Solid dots and solid line are for Diatomaceous earth (DE) pellets. The numbers over each dot represent the binder concentration (0-4). The correlation coefficients (r), shown in the legends, are considered significant when higher than 0.875 (95 Percent Critical Value with a degree of freedom of 3)*

Final germination at 0.0 MPa with DE had a slightly positive (R = 0.289) correlation, whilst TL had a negative correlation (R = - 0.335). At -0.5 MPa, correlations were more markedly negative for both DE (R = - 0.505) and TL (R = - 0.718), suggesting that increased mechanical resistance proprieties of the pellet, especially under low water availability condition, tend to reduce final germination. However, the correlation was not significant in any of the cases (Figure 3.4).

The effect of mechanical integrity was more evident in germination speed (T50) for both powders and in both water potential scenarios. DE and TL at 0.0 MPa, and TL at -0.5 had a significant correlation coefficient ($R > 0.875$), whilst DE at -0.5, although having a regression similar to TL (Figure 3.4), was slightly below the significant threshold ($R = 0.833$). These show how the mechanical proprieties of a pellet have a significant effect in delaying germination but don't influence final germination as much. The effects of pellet mechanical integrity on germination speed is probably due to the mechanical resistance posed by the pelleting material to the protruding radicle (Govinden-Soulange and Levantard 2008). A similar phenomenon has been described for seed that present natural external structures, such as husks in grass florets, that are responsible for delayed germination due to mechanical constraint (Pedrini et al. 2018b).

### 3.3.4. *Trade-off between germination speed and mechanical stress resistance*

A trade-off analysis was performed on mechanical variables and germination responses that in the previous correlation analysis have shown a significant response to binder concentration: specifically, germination speed (T50) and mechanical stress resistance. Across powders and water availability, the optimal binder concentration values were similar, ranging from 2.19% to 2.41% (Figure 3.5). In both water availability scenarios, DE had a higher optimal binder concentration than TL. Higher binder concentration in DE (2.37% at 0.0MPa, and 2.41% at -0.5 MPa) resulted in higher predicted mechanical stress resistance, but slightly slower germination speed, compared with TL, where optimal binder concentration was lower (2.37% at 0.0 MPa, and 2.41% at -0.5MPa). Comparing water availability, there was no clear trend, with binder concentration increasing at lower water potential in DE while decreasing for TL (Figure 3.5).

This trade-off analysis was focused on the binder concentration outcome that would allow for best mechanical resistance with the least effects on germination speed. Pellet-induced delays in germination have often been reported in the literature (Govinden-Soulange and Levantard 2008). However, this effect is not always considered negatively. For example, delayed germination in *Trifolium repens* might prevent seeds from emerging too early in the season, where climatic conditions are not suitable for growth (Esfahani and Shariati 2006). In some scenarios higher mechanical integrity

proprieties are needed, to avoid pellets crumbling during handling or sowing (Hill 1999). In either case, the trade-off analysis allows for the calculation of the binder concentration needed to reach the desired mechanical stress resistance or the expected germination speed.

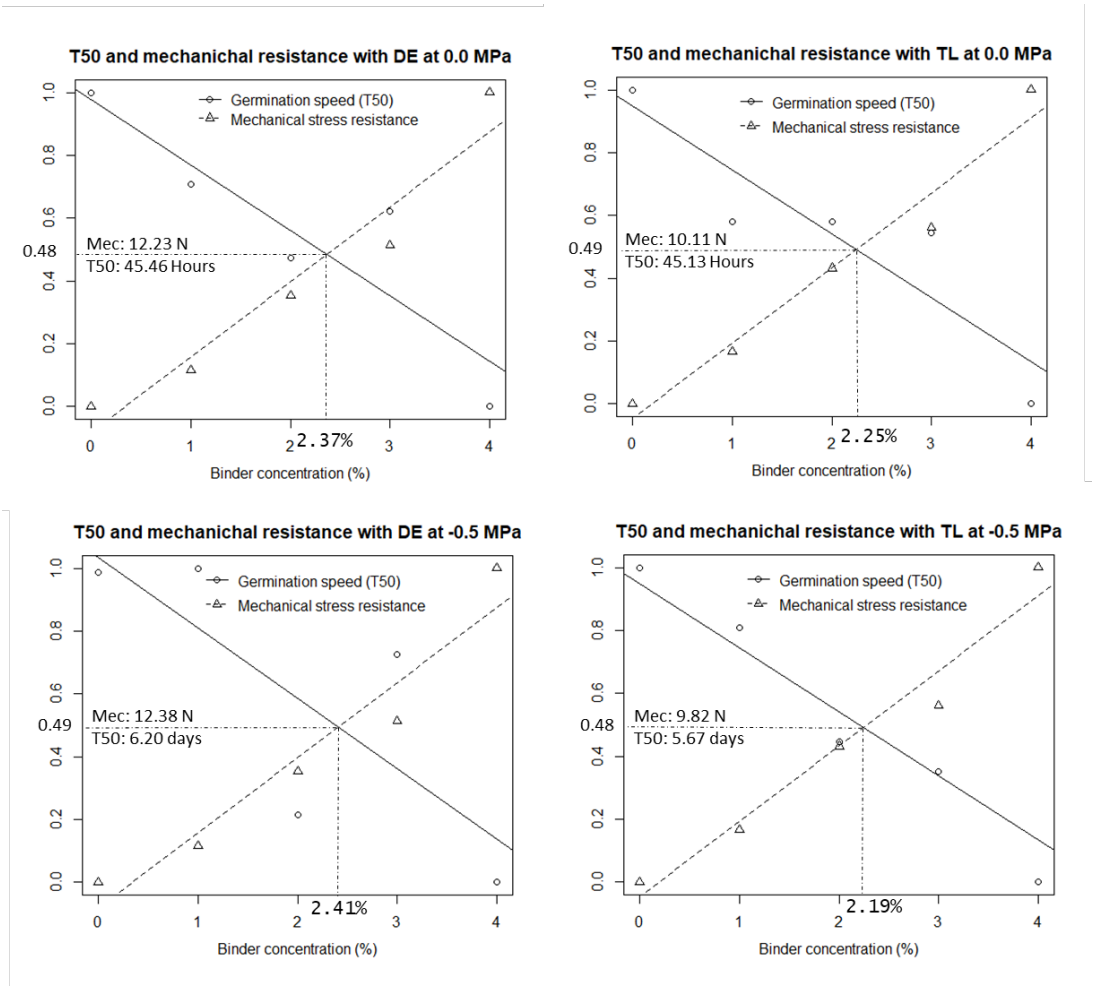



*Figure 3.5:Trade-off analysis graph for the optimal binder concentration percentage that maximises mechanical resistance whilst minimising delay in germination. The lines in each graph are the linear regression of normalised germination speed (dots, solid line) and mechanical stress resistance (triangles, dotted line) on binder concentration. The intersection of the two regression line represents the trade-off point. The X value of the trade-off point in the optimal binder concentration, whilst the Y value is the resulting normalised value for mechanical resistance and T50. Next to the X intersection point, the re-converted values for mechanical resistance (Mec) and germination speed (T50) are reported.*

In the present study, we have evaluated binder concentration up to 4% because at higher concentrations the binder was too viscous to be delivered with the employed equipment. At this concentration range (0-4%) the mechanical resistance response to binder concentration could be fitted in a linear regression. However, if tested at higher concentrations, a dose-response curve would probably be more appropriate, with mechanical resistance reaching a plateau at a specific point, after which further increase in binder concentration is no longer functional. A similar behaviour could be

expected for germination speed. Such assumptions have not been tested, but if confirmed might improve the precision of the model and extend its applicability.

## 3.4. Conclusion

This study has highlighted how physical proprieties of the pellet can influence seed germination in response to the mechanical integrity of the pellet other than its water absorption characteristics.

This behaviour was observed for the test species tomato, using one binder and two powders. Further experiments are required to understand if other species and material combinations would provide similar responses, validating the trade-off model and allow for its broader application across most of seed pelleting applications. However variables such as particle size distribution for fillers (Grellier et al. 1999), and water holding capacity (hydrophilic or hydrophobic) of the binder (Kavak and Eser 2009), will change water imbibition behaviour in a way that could impact germination. A variable that was not taken in consideration in this study, but might play a role in germination of pelleted seed, is oxygen availability to the seed through the pelleting layers (Sachs et al. 1981). To optimise the effectiveness of seed pelleting, research needs to investigate how these variables affect seed germination so as to improve control of the mechanical proprieties of the pellet and interactions with the physiological capability of the seed.

## 3.5. Bibliography

Abdel-Halim ES (2014) Chemical modification of cellulose extracted from sugarcane bagasse: Preparation of hydroxyethyl cellulose. Arab J Chem 7:362–371. doi: 10.1016/j.arabjc.2013.05.006

Almeida A De, Couto HTZ Do, Almeida ÁF De (2010) Are camouflaged seeds less attacked by wild birds? Sci Agric 67:170–175. doi: 10.1590/S0103-90162010000200007

Almeida C De, Rocha SCDS, Razera LF (2005) Polymer coating, germination and vigor of broccoli seeds. Sci. Agric. 62:221–226

Arshadi J, Asgharipour MR (2011) The Effects of Seed Size on Germination and Early Seedling Growth of Pelleted Seeds. J Appl Sci Res 7:1257–1260

Babu BNH, Kempegowda ML, Kalappa VP, Naik VR (2005) Comparative Evaluation of Different Pelleting Materials on Seed Performance of Frenchbean and Greengram. Karnataka J Agric Sci 18:32–35

Dow (2005) Cellosize - Hydroxyethyl cellulose. https://www.dow.com/assets/attachments/industry/building_construction/cellosize_brochure.pdf

Esfahani MN, Shariati M (2006) The use of pelleting in order to delay germination on Trifolium repens. Pakistan J Biol Sci 9:893–897

Fields PG, Allen S, Korunic Z, et al (2002) Standardized testing for diatomaceous earth. Proc 8th Int Work Conf Stored-Product Prot 1–13

Gevrek MN, Atasoy GD, Yigit A (2012) Growth and Yield Response of Rice ( Oryza sativa ) to Different Seed Coating Agents. Int J Agric Biol 14:826–830

Govinden-Soulange J, Levantard M (2008) Comparative studies of seed priming and pelleting on percentage and meantime to germination of seeds of tomato ( Lycopersicon esculentum Mill .). African J Agric Res 3:725–731

Grellier P, Riviere LM, Renault P (1999) Transfer and water-retention properties of seed-pelleting materials. Eur J Agron 10:57–65

Halmer P (2008) Seed Technology and Seed Enhancement. Acta Hortic 771:17–26

Halmer P (2000) Commercial seed treatment technology. In: I. Black M, Bewley JD (eds) Seed Technology and its biological basis. Sheffield Academic Press, Sheffield, pp 257–283

Hill HJ (1999) Recent Developments in Seed Technology. J New Seeds 1:105–112. doi: 10.1300/J153v01n01_09

ISTA (2007) Internationl Rule for Seed Testing

Kavak S, Eser B (2009) Influence of polymer coatings on water uptake and germination of onion (Allium cepa L. cv. Aki) seeds before and after storage. Sci Hortic (Amsterdam) 121:7–11. doi: 10.1016/j.scienta.2009.01.020

Michel BE, Kaufmann MR (1973) The Osmotic Potential of Polyethylene Glycol 6000. Plant Physiol 51:914–916. doi: 10.1104/pp.51.5.914

Murata MR, Zharare GE, Hammes PS (2008) Pelleting or Priming Seed with Calcium Improves Groundnut Seedling Survival in Acid Soils. J Plant Nutr 31:1736–1745. doi: 10.1080/01904160802324787

Nuyttens D, Devarrewaere W, Verboven P, Foqué D (2013) Pesticide-laden dust emission and drift from treated seeds during seed drilling: A review. Pest Manag Sci 69:564–575. doi: 10.1002/ps.3485

Pedrini S, Bhalsing K, Cross AT, Dixon KW (2018a) Protocol Development Tool (PDT) for seed encrusting and pelleting. Seed Sci Technol 46:393–405. doi: 10.15258/sst.2018.46.2.21

Pedrini S, Lewandrowski W, Stevens JC, Dixon KW (2018b) Optimizing seed processing techniques to improve germination and sowability of native grasses for ecological restoration. Plant Biol. doi: 10.1111/plb.12885

Pedrini S, Merritt DJ, Stevens J, Dixon K (2017) Seed Coating: Science or Marketing Spin? Trends Plant Sci 22:106–116. doi: 10.1016/j.tplants.2016.11.002

R Core Team (2015) R: A Language and Environment for Statistical Computing.itle

Ritz C, Baty F, Streibig JC, Gerhard D (2015) Dose-response analysis using R. PLoS One 10:1–13. doi: 10.1371/journal.pone.0146021

Ritz C, Streibig JC, Ritz, C. & Streibig JC (2005) Bioassay Analysis using R. J Stat Softw 12:1–22. doi: 10.18637/jss.v012.i05

Sachs M, Cantliffe DJ, Nell TA (1981) Germination studies of clay-coated sweet pepper seeds. J Am Soc Hortic Sci 106:385–389

Sanchez PL, Chen M, Pessarakli M, et al (2014) Effects of temperature and salinity on germination of non-pelleted and pelleted guayule (Parthenium argentatum A. Gray) seeds. Ind Crops Prod 55:90–96. doi: 10.1016/j.indcrop.2014.01.050

Scott JM (1989) Seed coatings and treatments and their effects on plant establishment. Adv Agron 42:43–83

Somrat N, Sawadeemit C, Vearasilp S, et al (2017) Effects of different binder types and concentrations on physical and antioxidant properties of pelleted sweet corn seeds. Eur Food Res Technol 1–8. doi: 10.1007/s00217-017-2979-y

Song Y, Huang Y, Wang X, et al (2014) Preparation and Control Efficiency of Seed Coating Agent by Antagonistic Actinomycetes Against Clubroot. J Agric Sci 6:132–139. doi: 10.5539/jas.v6n3p132

Sooter CA, Millier WF (1978) The Effect of Pellet Coatings on the Seedling Emergence from Lettuce Seeds. Trans Am Soc Agric Biol Eng 21:1034–1039

stackoverflow.com (2016) Finding where two linear fits intersect in R. https://stackoverflow.com/questions/7114703/finding-where-two-linear-fits-intersect-in-r. Accessed 16 Nov 2018

Stratistics (2017) Seed Coating Materials - Global Market Outlook (2017-2023). https://www.strategymrc.com/report/seed-coating-materials-market-2017. Accessed 6 Nov 2018

Taylor AG, Allen PS, Bennett M a., et al (1998) Seed enhancements. Seed Sci Res 8:245–256. doi: 10.1017/S0960258500004141

Taylor AG, Grabe DF, Paine D. (1997) Moisture content and water activity determination of pelleted and film-coated seeds. Seed Technol 19:24–32

Taylor AG, Harman GE (1990) Concepts and Technologies of Selected Seed Treatments. Annu. Rev. Phytopathol. 28:321–339

Texasgateway.org (2007) 95 Percent Critical Values of the Sample Correlation Coefficient Table. https://www.texasgateway.org/resource/126-outliers#eip-idm31993488. Accessed 16 Nov 2018

Wang EW (2012) Research on the Effect of Rape Seed Germination Based on Environment Friendly Seed Coating Mixing Different Potassium Fertilizers. Appl Mech Mater 246–247:648–652. doi: 10.4028/www.scientific.net/AMM.246-247.648

Zuffo AM, Zambiazzi EV, Carvalho MLM de, et al (2017) Quality of pelleted and bare lettuce seeds at different temperatures. Aust J Crop Sci 11:338–342. doi: 10.21475/ajcs.17.11.03.pne352

# 4. Optimizing seed processing techniques to improve germination and sowability of native grasses for ecological restoration*

## 4.1. Abstract

1) Grasslands across the globe are undergoing expansive degradation due to human impacts and climate change. If restoration of degraded native grassland is to be achieved at the scale now required, cost effective means for seed-based establishment of grass species is crucial. However, grass seeds present numerous challenges associated with handling and germination performance that must be overcome to improve the efficiency of seeding. Previous research has demonstrated that complete removal of the palea and lemma (husk) maximises germination performance hence we investigated the effects of complete husk removal on seed handling and germination on four temperate Australian grass species.

2) Three techniques were tested to remove the husk - manual cleaning, flaming, or acid digestion (the latter two followed by a manual cleaning step), these techniques were refined and adapted to the selected species and germination responses compared.

3) The complete removal of the husk improved seed handling and sowability for all species. Germination was improved in *Microlaena stipoides* by 19% and in *Rytidosperma geniculatum* by 11%. Of the husk removal methods tested, flaming was detrimental to seed germination and fatal for one species (*R. geniculatum).* Compared to manual cleaning, sulphuric acid improved the overall efficacy of the cleaning procedure and increased germination speed (T50) in *A. scabra*, *C. truncata* and in *M. stipoides* and improved final germination in *R. geniculatum* by 13%.

4) The seed processing methods developed and tested in the present study can be applied to grass species that present similar handling and germination performance

* This chapter was published on August 2018: Pedrini S, Lewandrowski W, Stevens JC, Dixon KW (2018) Optimizing seed processing techniques to improve germination and sowability of native grasses for ecological restoration. Plant Biol. doi: 10.1111/plb.12885

**Authors' contribution**: Simone Pedrini conceived the study, performed the processing treatment and wrote the manuscript. Simone Pedrini and Wolfgang Lewandrowski analysed and interpreted the data. Wolfgang Lewandrowski, Jason Stevens and Kingsley Dixon provided editing and revision to the manuscript. Simone Pedrini coordinated the publication process.

impediments. These and other technological developments (seed coating and precision sowing) will facilitate more efficient grassland restoration at large scale.

## 4.2. Introduction

Grasslands are distributed across a third of the global terrestrial area, covering a surface exceeding 50 million square kilometres (White et al. 2000). Given their dominance, grasslands provide important ecosystem services sequestrating atmospheric carbon, controlling erosion, and supporting livestock for meat and dairy production (O'Mara 2012). Yet, between 7.5% and 16% (Conant 2010) of grassland ecosystems are undergoing degradation mostly due to overgrazing, land clearing, soil erosion, exotic species invasion and climate change (O'Mara 2012). Ongoing degradation has decreased net primary production in grasslands worldwide by an estimated 50% (Gang et al. 2014). These changes are predicted to negatively impact biodiversity, and affect the livelihood of approximately 1.5 billion of people who rely on natural ecosystem services for sustenance (Bai et al. 2008). As such, improved management is needed to recover many altered grasslands. When degradation reaches a state where ecosystems can no longer recover naturally, intervention such as ecological restoration is required to establish a pre-disturbance recovery trajectory (Society for Ecological Restoration International Science & Policy Working Group 2004; McDonald et al. 2016).

Although the use of nursery grown seedlings or young plants is common for the restoration of small areas, currently the most cost-effective way to re-establish large plant populations is through seeding (Merritt and Dixon 2011). However, native seed quantity and quality factors limit deployment of native seed in restoration (Gibson-Roy et al. 2006). Recent studies have highlighted a major recruitment bottleneck in restoration programs, with establishment from seeds rarely exceeding 10% (James et al. 2011; Larson et al. 2015; Lewandrowski et al. 2017). While temperature and soil moisture are fundamental factors regulating germination processes, seed viability, germinability (Gibson-Roy et al. 2007), and precision seed delivery to a suitable microsite (Chambers and Macmahon 1994) are essential to overcome establishment failures (Merritt and Dixon 2011).

In native seed mixes for grassland restoration, grass seeds can represent up to 90% of the mix weight (Mitchley et al. 2012). Grass florets present numerous challenges

associated with handling and germination performance that must be overcome to improve the efficiency of seed based restoration (Stevens et al. 2015; Guzzomi et al. 2016; Erickson et al. 2016b; Lewandrowski et al. 2017). The native seed industry in Australia has focused on developing customised seeding equipment to overcome sowability problems associated with handling grass seed, (Loch et al. 1996) with limited attempts to tackle the source of the problem – the irregular shape, appendages and indumenta associated with native grass seed.

Each floret contains a single seed (caryopsis) enclosed in the covering structures palea and lemma (hereafter referred as husk), for improving anemochorous and epizoochorous dispersal (Ernst et al. 1992) and in some cases, seed orientation, and/or self-burial after dispersal (Humphreys et al. 2011). While providing functional benefits for dispersal in natural recruitment, under artificial sowing conditions, the husk affects germination performance by imposing both a mechanical barrier to the developing embryo during germination (Bewley et al. 2013; Farley et al. 2013; Erickson et al. 2016b) but also affects germination speed by controlling oxygen and water uptake into the seed (Huarte et al. 2007). The floret-imposed reduction of germination could therefore limit the successful establishment of a grass seedling and the overall outcome of using seed in a restoration program. Complete removal of the husk has been shown to significantly improve germination performance (Adkins and Simpson 1988; Bewley et al. 2013; Erickson et al. 2017). For example, Ma *et al.* (2008) show an increase of 20% in germination after seed is removed from the external floret structures in *Leymus chinensis*.

Mechanical de-awning or de-husking by threshing/sizing, rubbing and agitation (Loch et al. 1996) has been tested on Australian grass species, however, damage to embryo was found to substantially decrease seed viability (Whalley and Jones 1996). Different and less invasive techniques have been recently developed for reducing the floret using flash flaming (Guzzomi et al. 2016) or sulphuric acid (Stevens et al. 2015) that focus on partial removal of the floret structures and appendages. Flash flaming uses the intermittent exposure of seed to a high intensity flame that leads to scorching and removal of floret appendages (hairs and awns) without mechanical or physiological damage to the embryo and seed. This method, tested on the Australian grass *Triodia wiseana*, has improved both seed germination and handling (Guzzomi et al. 2016). Sulphuric acid scarification, usually applied for treating physically dormant seed (Baskin and Baskin 2014) was applied by Stevens *et al.* (2015) for managing

impediments to germination in four grass species, resulting in overall improved seed handling and for one species with poor germination performance (*Microlaena stipoides*) a 25% improvement in germination, most likely due to removal of mechanical constraint of the husk on the emerging embryo. However, in two of the test species, *Rytidosperma caespitosum* and *Chloris truncata,* acid digestion provided no improvement in germination, when treated at 50% sulphuric acid, with a drop in germination at higher acid concentrations. Although both techniques have shown some promising results for improving seed use in grassland restoration, to date, these approaches have not been employed for the complete removal of the husk, considered by many as a means to optimise germination potential (Adkins and Simpson 1988; Bewley et al. 2013; Erickson et al. 2017). Moreover, the advantages of husk removal have not been tested as a means for improving sowability.

This study therefore aimed to: (1) identify the degree to which the complete removal of the husk affects seed germination, handling, sowability and consistency in seed delivery on four grass species and (2) compare different husk removal techniques to determine the impact on germination and processing efficiency.

By resolving which technique improves precision seeding capability while not decreasing germination performance, will have positive implications for grassland restoration through improving the success of direct seeding programs.

## 4.3. Methods

### 4.3.1. *Species selection and characterisation*

Four species of native Australian grasses (*Austrostipa scabra* (Lindl.) S.W.L.Jacobs & J.Everett *, Chloris truncata* R.Br., *Microlaena stipoides* (Labill.) R.Br. var. Griffin and *Rytidosperma genicula* (J.M.Black) Connor & Edgar var. Oxley), commonly used in revegetation and pasture establishment in temperate Australia were tested on the basis of their differing seed morphologies. According to Loch *et al.* (1996), the selected species belong to four different 'seed' categories: *A. scabra* has a single dispersal unit with hygroscopic awns; *C. truncata has a* single dispersal unit with rigid awns and papery husk; *M. stipoides* seed comes in a single dispersal unit with rigid awns and tough husk and *R. geniculatum* florets have a single dispersal unit with surface hairs (Figure 4.1).

Freshly harvested seeds were obtained from Native Seed Pty Ltd (Cheltenham, Victoria, Australia). Prior to processing, seeds were stored on open shelving at ambient conditions of 20 - 25° C and relative humidity at 40-50% for three months. All seed batches were assessed for seed fill, density, and floret weight. Seed fill was evaluated by examining five replicates of 50 seeds of each species using X-ray (Faxitron MX-20 X-ray cabinet, Tucson, USA), set at 22 kVa with a 10 second exposure time. Filled florets were then grouped into five batches of 50 to determine floret weight and number of florets per gram. A known quantity of florets (50 or 100 g) was placed in a 2 L graduated beaker to assess the volume (ml). The batch was then cleaned (using the sulphuric acid digestion method) and the resulting cleaned caryopses were weighed, the volume assessed and bulk density calculated.

### 4.3.2. *Sowability and seed delivery consistancy test*

The seed industry standard approach to test flowability, pouring seed through a funnel and recording time for passage (Davies et al. 1995) was not appropriate as the florets could not flow through the funnel because of bridging. Therefore, rather than assessing flowability, we evaluated the sowability and consistency in seed delivery using a hand-operated Earthway precision seeding machine (Parish and Bracy 2004), with different metering plates to accommodate the differing size and shape of caryopses/florets of the study species (Table 4.3). For the smaller caryopses of *C. truncata and R. geniculatum* a customised metering plate was designed by drilling 18 equidistant, 2mm wide holes on a blank metering plate. The Earthway seeder was locked in a standing position, seed loaded into the hopper and the front wheel was manually rotated to simulate the seeding operation. Seed dispensed into the sowing tube were collected. The wheel was rotated for 1, 2, 3 and 3.6 cycles according to the numbers of holes on the metering plate, in order to assess the delivery through 36 holes. The process was repeated five times on florets and caryopses for each species. Sowability was considered as the total number of seed deposited and consistency in delivery as the standard error percentage relative to the average of the replicates.

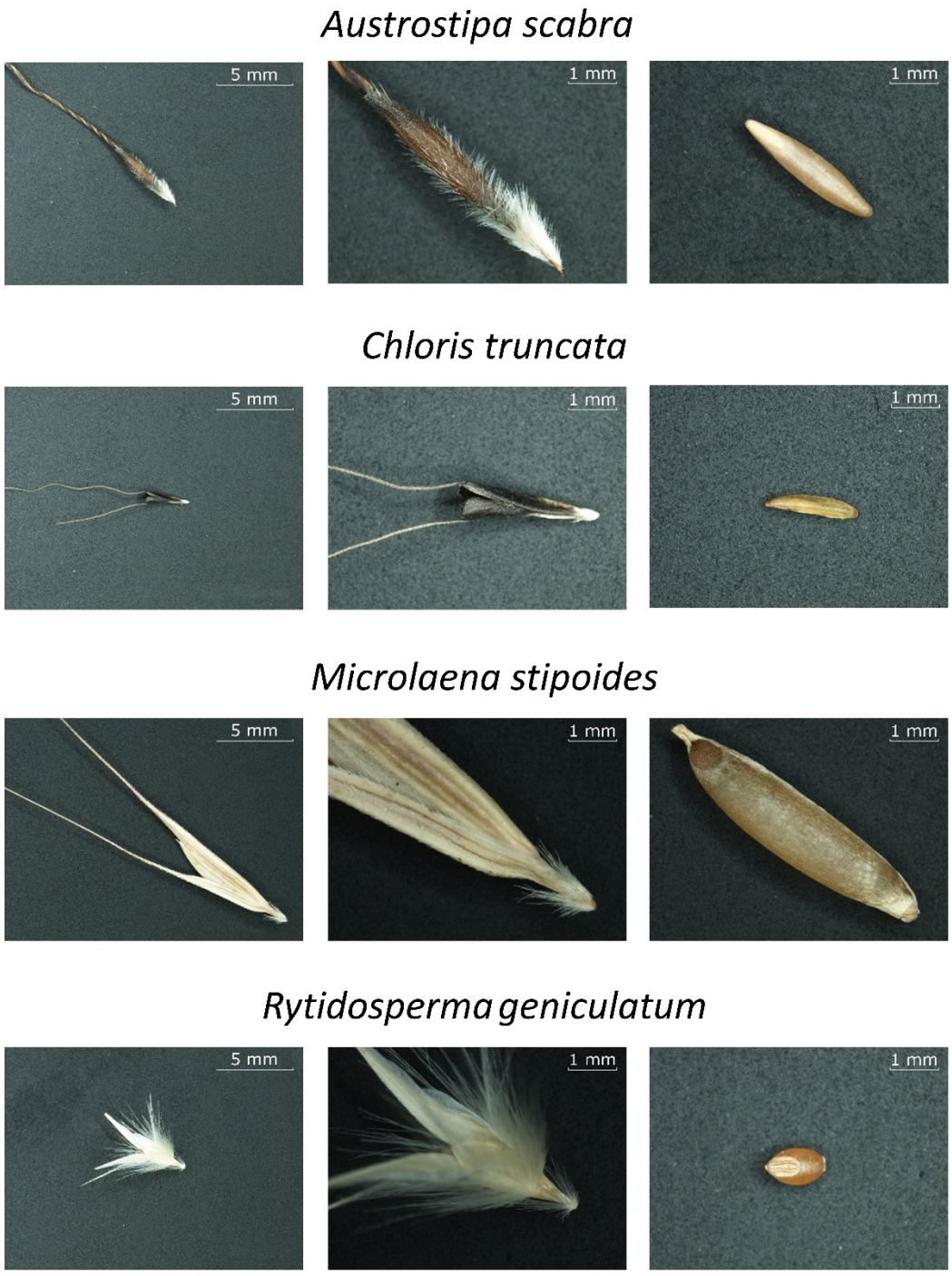



*Figure 4.1: Floret and caryopsis of the test species*

### 4.3.3. *Mechanical cleaning*

In the mechanical cleaning process (hereafter referred as MC) caryopses were extracted from 5-10 g seed lots using direct abrasion. This involved rubbing florets

across a ribbed, rubber mat with constant light force to avoid seed damage, especially on spindle-shaped seed such as *M. stipoides* and *A. scabra*, where the seed could easily be broken if subjected to excessive pressure (Loch et al. 1996). Once husks were separated, seed was purified by differential sieving. The remains were cleaned with zig-zag air flow separator (Selecta Machinefabriek BV, Enkhuizen, Netherlands). If unclean seed were still found, they were re-processed as above. Cleaning time, seed weight, 1000 seed weight and volume were recorded and density calculated (weight/volume).

### 4.3.4. *Flaming*

The process of flaming (hereafter referred as FL) was performed using a rotor-RP14DB® vertical drum coater (BraceWorks Automation and Electric, Lloydmin- ster, SK, Canada) adapted with a BernzOmatic UL2317 propane torch, (BernzOmatic, Columbus, OH, U.S.A.), installed on a patented seed ablation device (Ling et al. 2015). Florets were exposed to a continuous flame methodology described by Guzzomi et al. (2016), however, this approach was modified by increasing the flame-exposure time and adding a further step of mechanical cleaning to completely remove the husk. Florets were treated in the flame ablator for differing periods of time depending upon the degree of floret ablation required (Table 4.1). Subsamples of seed were removed during the flaming cycles to assess the degree to which confounding appendages and bracts were weakened sufficiently to allow for more effective subsequent mechanical treatment.

### 4.3.5. *Acid digestion*

Acid digestion (hereafter referred as AC) was performed by submerging 50 g batches of floret in a 1.4 L 50% sulphuric acid solution (ACS reagent grade $H_2SO_4$, Sigma-Aldrich, St Louis, USA). Florets were immersed for varying time to achieve the desired level of floret digestion based initially on intervals of Stevens et al. (2015) and by visually assessing of the level of floret degradation.

After removal from the acid solution, digested florets were neutralised in 8.4 g $L^{-1}$ sodium bicarbonate ($NaHCO_3$, Sigma-Aldrich, St Louis, USA) solution for 5 minutes. Florets were then rinsed under tap water for two minutes and then dried in a Food Lab™ Electronic Dehydrator at 35° C for three hours (Sunbeam, Sydney, Australia). The resulting dry residue of digested husk and caryopses was subjected to mechanical

cleaning process to completely separate the caryopses from residual appendages and debris.

*Table 4.1: Exposure time to different treatment per species. For flaming corresponds to the time the seed were continuously exposed to flames. The time was determined by assessing the structural integrity of the floret and its resistance to mechanical cleaning. Shorter exposures were tested on R. geniculatum, when tested times proved to be fatal for the seed. Acid digestion was assessed upon visual evaluation of floret degradation and exposure of the caryopses.*

| SPECIES | FLAMING | ACID DIGESTION |
|---|---|---|
| ***Austrostipa scabra*** | 5 - 10 - 15 min | 90 min |
| ***Chloris truncata*** | 5 - 10 - 15 min | 30 - 60 - 90 min |
| ***Microlaena stipoides*** | 15 - 30 - 60 min | 60 - 90 min |
| ***Rytidosperma geniculatum*** | 5 - 10 - 15 min (15-30-60 sec) | 10 - 20 - 30 min |

### 4.3.6. *Germination*

Twenty five florets/caryopses were placed in each Petri dish and replicated four times per treatment. Sown seed was incubated on moistened filter paper in Petri dishes in a Biosyn 6000 OP (Contherm, Korokoro, New Zealand) at 20° on a 12/12 hours light-dark cycle. To avoid desiccation, the dishes were sealed with transparent cling wrap and 2 ml of water were added weekly to maintain a moist but not wet environment. Germination was recorded when the radicle was at least 1mm in length. The germinated seeds were removed once scored. Germination was recorded daily for the first four days and then at 6, 8, 12 and 21 days. At the end of the experiment, the remaining seed were tested for viability via a cut test and inspection for presence of an intact, embryo and endosperm.

The first set of germination tests was performed to identify differences among untreated seed and seed with the husk removed manually. Complete husk removal with flaming or acid digestion were tested for different exposure times (when applicable). The treatment exposure times that resulted in the best germination outcome, were then compared between manual, flaming and acid digestion processing methods.

### 4.3.7. *Data analysis*

The mean and standard error were calculated for the sowability test. To determine the consistency of the sowing process, standard error percentage relative to the average was also considered.

$$(100/\varepsilon) * se$$

"$\varepsilon$" is the average of seed deposited through a seeding round (five replicates) and "*se*" is the standard error for that round.

To determine cumulative germination response over time for the various seed treatment tested, data analysis was performed on the software R (R Core Team 2015). Non-linear regression models were fitted with the function "drm" of the "DRC" package (Ritz et al. 2005, 2015). A three parameter log-logistic model was used:

$$f(x) = \frac{gmax}{1 + \left(\frac{x}{T50}\right)^b}$$

The parameters are: (b) slope curvature, (gmax) final germination and (T50) germination speed, intended as time (days) required to reach half of the final germination. Parameter comparison on final germination and germination speed were then performed to assess differences among treatment (significance $p < 0.05$).

## 4.4. Results

### 4.4.1. *Seed characteristics*

X-ray analysis of unprocessed seed, revealed that floret fill ranged from 96.4 ± 1.8% seed fill in *A. scabra* to 13.2 ± 1.9% in *C. truncata*. At 45-50% RH, floret material constitutes most of the weight in all seed lots examined, from 60% in *M. stipoides* up to 99% in *C. truncata*. The difference in density between caryopses and floret ranged from 20-fold increase in *M. stipoides*, to 280-fold in *C. truncata* (Table 4.2).

### 4.4.2. *Sowability and seed delivery consistancy test*

Seed deposition (seed counted / number of holes) was lower for florets than for manually cleaned seed (Table 4.3). For uncleaned *A. scabra,* the florets were so tangled

that no seed passed through the metering plate, regardless of the hole size. Seeding consistency, considered as relative standard error (% se), was better for caryopses (with a maximum of 3.16% in *A. scabra*) than in floret that range from 10% (*R. geniculatum*) to 40.8% (*A. scabra*). Upon visual assessment no damage or cracking was detected in the caryopses.

### 4.4.3. *Seed processing treatment*

Manual cleaning was performed on 10 g batches of seed for all the species and ranged from 45-50 minutes to achieve cleaned caryopses. The rubbing, sieve sorting and air flow separation steps were repeated multiple times before a batch of completely cleaned caryopses could be obtained. For *A. scabra* and *M. stipoides* there was some damage (fractured or shattered) to seed during the cleaning procedure.

Flaming allowed for the processing of 20 g of seed in a single batch, while 50 g batches of seed were used for seed acid seed digestion. Both treatments failed to completely remove the husk in the four species tested and further manual cleaning was required to 'dehusk' the caryopses. However, floret exposure to flame and acid weakened the structural integrity of the floret structure and allowed for a complete removal of the husk in a single mechanical cleaning cycle, (that was performed between 5 and 10 minutes, according to the batch size).

*Table 4.2: Characterization of floret and cleaned caryopsis in four Australian grass species. Seed fill in the floret was recorded in 5 batches of 50 seeds randomly selected from the seed lot. Volume and density of a known quantity of floret (weight) were recorded from a subsample, directly collected from the original seed lot, without any cleaning or sorting process. This subset was then cleaned to caryopsis using the sulphuric acid method and the weight, volume and density were recorded. TSW (1000 seed weight) was calculated from 5 batches of 50 filled floret, or 50 caryopses.*

| Species | | Seed fill (% ± st. err) | Weight (g) | Volume (L) | Density (kg $m^{-3}$) | TSW (g ± st.err) |
|---|---|---|---|---|---|---|
| *Austrostipa* | floret | 96.4 ± 1.8 | 100 | 1.02 | 0.98 | 1.598 ± 0.041 |
| *scabra* | caryopsis | - | 27.3 | 0.0425 | 642.3 | 0.760 ± 0.021 |
| *Chloris* | floret | 13.2 ± 1.9 | 50 | 1.40 | 35.7 | 0.151 ± 0.004 |
| *truncata* | caryopsis | - | 2.54 | 0.005 | 508.0 | 0.123 ± 0.002 |
| *Microlaena* | floret | 62.0 ± 2.3 | 100 | 1.20 | 83.3 | 4.818 ± 0.196 |

| | | | | | | |
|---|---|---|---|---|---|---|
| *stipoides* | caryopsis | - | 41.63 | 0.064 | 650.4 | 3.688 ± 0.058 |
| *Rytidosperma* | floret | 77.2 ± 3.3 | 100 | 1.80 | 55.5 | 0.783 ± 0.016 |
| *geniculatum* | caryopsis | - | 30.05 | 0.0365 | 823.2 | 0.347 ± 0.007 |

*Table 4.3: Direct seeding results (Earthway Precision Garden Seeding machine) comparing floret and cleaned seed. This test was performed simulating a sowing run by manually rotating the metering plate so that the seed in the hopper is picked up by 36 holes. Every simulation was run five times for each species for both florets and caryopses. In the "Metering plate" column, the code of the plate used and the number of holes on the plate are indicated. "Ø" is the diameters of the holes in mm. "Rot" is the number of rotation performed in order to run a total of 36 holes. "Sowability " is the number of floret or caryopsis that were delivered through the run and "Consistency" is the relative standard error relative to the average of the replicates* $(100/\varepsilon) * se$*. Higher the value, less consistent is the delivery. Deposition rate is the average number of seeds picked up by a single hole.*

FLORET

| Species | Metering plate | Ø mm | Rot | Sowability | Consistency | Deposition |
|---|---|---|---|---|---|---|
| *A. scabra* | (1002-14) 10 holes | 13 | 3.6 | - | - | - |
| *C. truncata* | (1002-5) 12 holes | 5 | 3 | 3.2 ± 1.31 | 40.89 | 0.08 |
| *M. stipoides* | (1002-14) 10 holes | 13 | 3.6 | 10.6 ± 2.38 | 22.42 | 0.29 |
| *R. geniculatum* | (1002-5) 12 holes | 5 | 3 | 4 ± 0.4 | 10 | 0.11 |

CARYOPSIS

| Species | Metering plate | Ø mm | Rot | Sowability | Consistency | Deposition |
|---|---|---|---|---|---|---|
| *A. scabra* | (1002-24) 36 holes | 3 | 1 | 97.4 ± 3.08 | 3.16 | 2.71 |
| *C. truncata* | (Custom) 18 holes | 2 | 2 | 86.4 ± 1.59 | 1.84 | 2.4 |
| *M. stipoides* | (1002-5) 12 holes | 5 | 3 | 70.8 ± 1.91 | 2.67 | 1.96 |
| *R. geniculatum* | (Custom) 18 holes | 2 | 2 | 86.4 ± 1.59 | 1.85 | 2.4 |

## 4.4.4. *Germination tests*

### *4.4.4.1.Floret vs caryopsis*

In *A. scabra* the manual removal of the husk provided no significant improvement in germination speed (T50) or final germination. Final germination in *C. truncata* was also

unaffected by husk removal, but T50 was reached 0.9 days faster ($P < 0.001$) for caryopses. A significant increase in both final germination (+ 17.4% $P < 0.001$) and germination speed (1.4 days faster $P < 0.001$) was recorded for cleaned seeds of *M. stipoides* and for *R. geniculatum,* T50 of cleaned seeds was attained 5.9 days faster ($P < 0.001$) and final germination was improved by 11.4% ($P < 0.05$) (Figure 4.2).

*4.4.4.2.Flaming*

When flaming was tested on *R. geniculatum*, all exposure times (Table 4.1) resulted in 0% germination. To understand what flame exposure time would not have been lethal, a set of further experiments was performed (Figure 4.3), and the exposure to flames was reduced to 15, 30, 60 seconds. Shorter exposures were not enough to improve the further manual processing. At 15-seconds, seed germination was reduced from almost 90% (MC) to less than 10% and no germination was recorded at 30 seconds or more. A similar effect was observed for *C. truncata*, with seed run through the flaming process for 5 minutes germinating at 45.6 ± 6.3% (MC: 70.2 ± 6.3%), reduced to 18.2 ± 3.6% after 15 minutes. For *A. scabra* and *M. stipoides,* there was no significant difference ($P < 0.05$) in final germination among flaming treatment exposure times.

*4.4.4.3.Acid digestion*

Sulphuric acid digestion in *C. truncata* for 60 and 30 minutes did not show any significant ($P < 0.05$) difference in germination although 60-minute exposure made subsequent cleaning faster and easier in terms of handling. For the 90-minute acid treatment, final germination decreased from 75.9% to 8.1%. Similarly, *R. geniculatum* germination was reduced for the longer exposure period (30-minute, final germination: 59.7 ± 2.6%), while 10 and 20-minute exposure had similar final germination responses of 85.5 ± 2.1 % and 88.9 ± 0.2%, respectively. In *M. stipoides* 90 minute exposure to acid significantly reduced ($p < 0.05$) final germination to 71.9 ± 1.6% and T50 to 2.1 ± 0.1 days compared to the 60-min treatment (final germination: 84.90 ± 1.57% and T50: 1.7 ± 0.1 days). Thirty minutes was not evaluated, as florets were still intact. *A. scabra* was not tested for different exposure times because 90-minute immersion proved to be effective in removing the husk while not limiting germination. Upon visual assessment, shorter times were insufficient to facilitate complete husk removal.

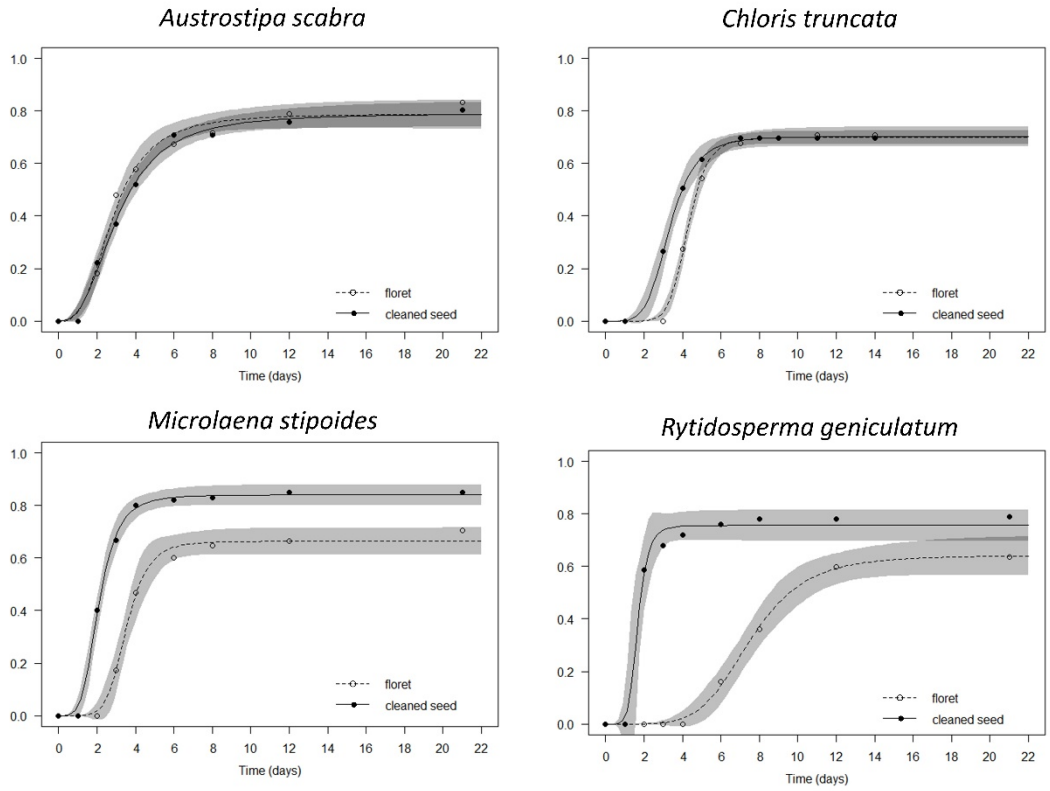



*Figure 4.2: Cumulative germination curve of floret and caryopsis. Results were estimated using a three parameter log-logistic curve. The lines represent the cumulative germination curve (proportion) over time. The dots indicate germination recorded on a specific day and shaded areas around the germination curves are the 95% confidence intervals.*

#### *4.4.4.4. Treatment comparison*

When exposure time that resulted in the best germination outcomes (Figure 4.3) for flaming and acid digestion were compared with manual cleaning, flaming was detrimental for the four study species ($P < 0.05$).

Compared to manual cleaning, final germination was significantly improved ($P < 0.05$) with acid digestion in *R. geniculatum* (AC: 88.9 ± 2.4%, MC: 75.6 ± 2.4%), and slightly, but not significantly, improved for *A. scabra*, *C. truncata* and *M. stipoides*. Germination speed (T50) was improved ($P < 0.001$) for *A. scabra* subjected to acid digestion (1.6 ± 0.1 days) than in manually cleaned (3.1 ± 0.2 days), similarly to *C. truncata* (AC: 3.0 ± 0.1 days, MC: 3.3 ± 0.1 days, $P < 0.05$) and *M. stipoides* (AC: 1.7 ± 0.1 days, MC: 2.1 ± 0.1 days, $P < 0.001$), However the opposite was observed for *R. geniculatum* (AC: 2.8 ± 0.2 days, MC: 1.7 ± 0.1 days, $P < 0.001$ ).

## 4.5. Discussion

### 4.5.1. *Germination response and improved handling following husk removal*

Compared to florets, caryopses showed higher germination for two of the species tested and provided for better handling, including ease of sowability and volume reduction across the four species tested.

The role of appendages in controlling germination in grasses is not fully understood, however according to Huarte *et al.* (2007) floret structures do not impede water intake by the caryopsis but could impose physical constraints that impair emergence of the radicle. This could explain the reduced final germination and speed in uncleaned *M. stipoides* and *R. geniculatum*, whose florets are harder and more rigid than the papery structures found in *C. truncata* and the thin floret of *A. scabra* where husk removal did not improve germination.

Seed germination speed (T50), increased by husk removal, provides important establishment benefits in terms of faster field germination to outcompete weed species (Vaughn and Young 2015). On the other hand faster germination could also expose the seed batch to a "false start" triggered by a small rainfall event that could lead to seedling mortality if soil moisture is not sustained (Loch et al. 1996). Fiel trial experiments could demonstrate the effects of increased germination speed on plant establishment and survival.

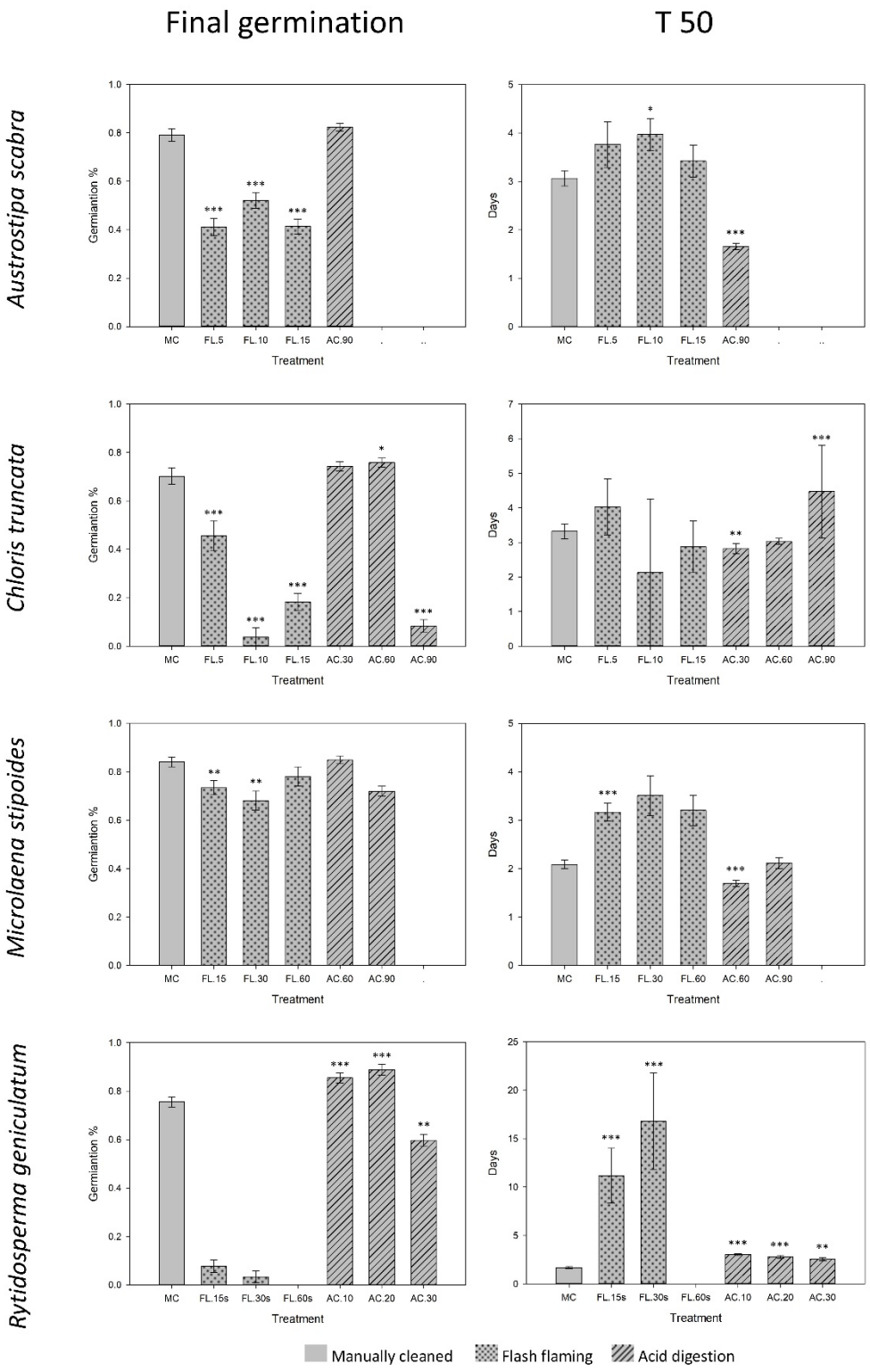



*Figure 4.3: Final germination percentage and germination speed as days required to reach 50% of the final germination (T50) with standard errors. MC = manual cleaning, FL = flaming, AC = acid digestion. The number next to the treatment code is the exposure time in minutes. If followed by "s" represent seconds of exposure. Significance indicated for each treatment compared to manual cleaning (* P<0.05, **P<0.01, ***P<0.001)*

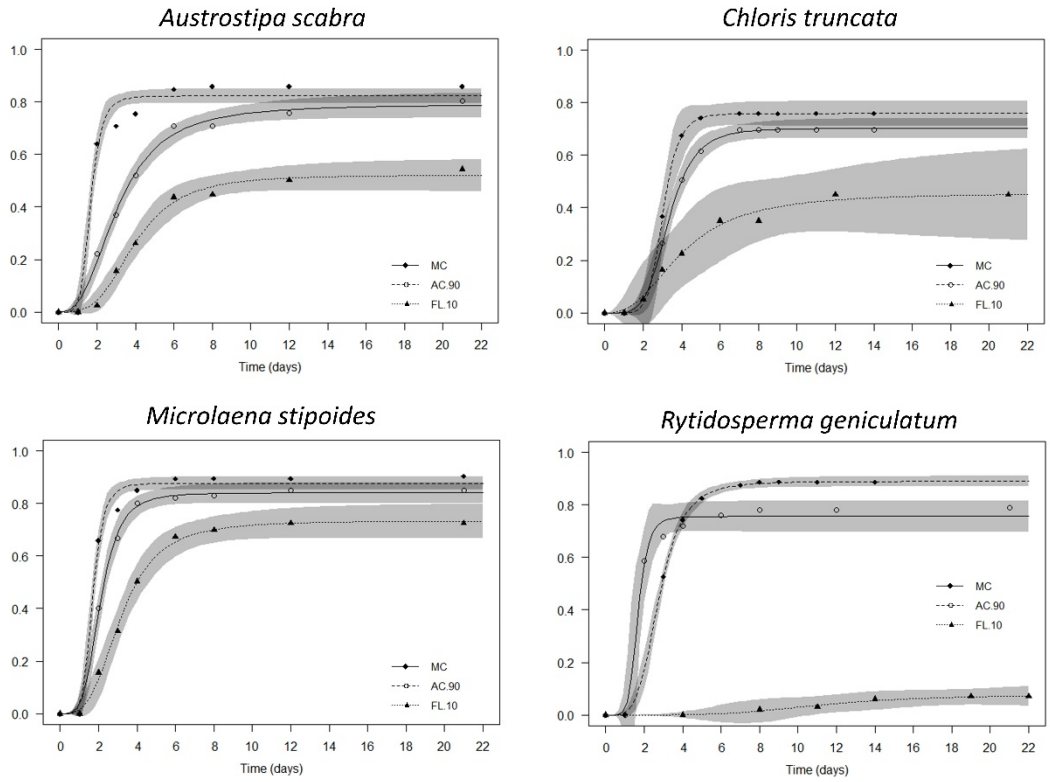



*Figure 4.4: Cumulative germination percentage curve of the three different seed processing methods, MC = manual cleaning, AC = acid digestion and FL = flaming. The exposure times for Flaming and Acid digestion shown represent the optimal germination response. Exposure time in minutes, are shown next to the treatment code (s = seconds). The lines represent the cumulative germination curve (percentage) over time. The dots show the germination recorded on a specific day and the shaded area around the germination curves represent the confidence intervals.*

Moreover, Cole *et al.* (2004) reported a decrease in germination for caryopsis of *R. geniculatum* over a 24 month storage time, compared to floret. This decrease might be due to seed damage during the mechanical husk removal process. Such damage could potentially be overcome with the treatment procedures used in this study, as they greatly reduce the intensity and duration of the mechanical process.

A significant advantage of husk removal is the increased bulk density of seed batches that economises on handling and storage space. Given the unreliability and unpredictability of wild harvesting of seed and the fluctuation in seed production even in controlled seed farms, the storage of large quantities of seed for many years is a good practice to compensate for potential production shortcoming (Broadhurst et al. 2016). Seed banks across the globe are now called to improve their storage capability to fill ecological restoration demand (Merritt and Dixon 2011). However, storing seed at the appropriate condition to optimise shelf life requires seed banking at controlled temperatures such as -18° C (Fao 2014) which is both expensive in the facilities and

the handling required if unprocessed grass seed is being stored. Thus, reducing seed volume as demonstrated in this study by orders of magnitude represents significant savings in space and handling that will improve the commercial viability of the native seed industry. One further benefit of cleaning seed to the caryopsis level is that it allows for the complete elimination of empty florets, that in some extreme cases like *C. truncata*, account for more that 85% of the total number of florets. Numerous arid zone grasses tend to have poor floret fill (Erickson et al. 2016a) hence the cleaning to caryopsis would allow complete removal of empty florets.

Seed delivery, as tested here, showed that caryopses flowed through the seeding mechanism easily and with increased consistency compared to intact florets with application to other mechanised seeding machines such as air seeder and drills. In grassland restoration, seed are usually delivered in mixes, and seed shape and density are crucial for ensuring delivery of different species at the desired densities. Floret structures are often responsible for bridging, clogging the sowing mechanism and blocking the delivery of seed (Waters et al. 2017). Bridging can be overcome by using bulking material (wood shavings, sand or vermiculite) vibrating hoppers, or rotating brushes that also improve flowability and overcomes the problem of interrupted seeding. However, when mixes contain seed of different size, shape and density, the vibrations in such machines can lead to seed sorting and subsequent bias when delivering mixed seed batches. The removal of the husk could decrease the differences in density among species allowing for a more even seed mixing and delivery that can be further improved through the use of seed encrusting and pelleting to produce seed of uniform shape, size and density (Pedrini et al. 2017).

### 4.5.2. *Seed processing methods: germination and logistics*

Our goal was to completely remove the husk from caryopses and the treatments imposed were comparatively assessed for efficiencies and impacts on subsequent seed germination success. Germination response, and seed processing efficacy, were improved in seeds processed through acid digestion, in comparison to flash-flaming, or manual cleaning. The reduced germination in seed cleaned manually is probably due to physical damage (Whalley and Jones 1996), caused by the vigorous rubbing action over a long time period required to crush the intact floret and expose the caryopsis.

Flaming, as defined in the current study (continuous flame for long durations followed by manual cleaning), had significantly detrimental effects on germination in the four

study species. This finding is in contrast with the increase in germination recorded for grass species where the goal was to reduce rather than remove husk via flash flaming (Guzzomi et al. 2016). Given the aim of completely removing the husk, and this result implies that the flaming technology might not be suited for this purpose.

Flash-flaming had different effects according to the species' floret structure. In seed with fluffy florets and abundant fine hairs such as *R. geniculatum* or a papery floret as in *C. truncata*, (Figure 4.4) the flaming process ignites the combustion of those appendices, setting alight to the mass of rotating seed for a few seconds. This floret burning event was fatal for *R. geniculatum* and substantially reduced germination in *C. truncata.* Exposure time was a decisive factor in reducing seed viability, with longer exposure resulting in lower germination outcomes. However, shorter exposure times were insufficient to remove or degrade the floret, providing little advantage in the final manual cleaning process, compared to the unflamed floret. This result suggests that the failure point could be in the flaming part of the process. However, in *M. stipoides* and in *A. scabra*, where there is no clear trend linking reduced germination with exposure time, the reduced germination could be due to the further step of mechanical cleaning rather than the flaming process itself.

Similarly, as previously shown by Stevens *et al.* (2015), germination responses following sulphuric acid digestion process are time dependent. Optimal exposure time envelopes could be identified for each species or, indeed any grass species by balancing exposure time that minimises viability loss while enhancing the ability to remove the husk (eg. *C. truncata, M. stipoides and R. geniculatum*).

Manual cleaning proved to be the most time consuming and labour intensive means for husk removal, while acid treatment was the most time effective. Both flaming and acid digestion allowed for the processing of higher quantities of seed, and although further manual cleaning was needed to completely remove husk debris and expose the caryopses, this additional operation required just one cleaning cycle (5-10 min) for a relatively large batch of seed (50-100 g) rather than multiple cleaning cycles (45-50 min) of small batches (10 g) of untreated florets. The seed flaming apparatus and acid digestion methodology tested here do not represent commercially applicable approaches, the acid treatment allowed for batch processing up to 100 g of floret compared to the 20 g for flaming and 10 g for manual cleaning. Although the entire acid digestion process of acid submersion (15-90 min), neutralising and rinsing (5 min),

drying (3 hours) and final manual cleaning (5-10 min), was longer than flaming, it required less active intervention by the operator, especially in the acid submersion and drying phases. In contrast, seed flaming required regular monitoring to guarantee constant flow of the seed inside the drum and avoid seed bridging on the side deflectors and consequent blockage and uneven distribution of the flame treatment.

### 4.5.3. *Potential for seed technology development and scaling up for restoration*

If further treatment to improve handling are needed after recovery of the caryopses, seed encrusting and pelleting would help standardise size, weight and density of different species, greatly improving the reliability of seed delivery to field sites (Pedrini et al. 2017). Moreover, those treatments could be used to deliver beneficial compounds such as germination promoters, stress and growth regulators and anti-predation agents (Greipsson 1999; Guan et al. 2014). As shown by Guzzomi *et al.* (2016) the partial removal of the husk improved the effectiveness of seed coating procedures. In cases where the floret structures are intricate (eg. *Austrostipa*), their removal is the best way to achieve a seed product capable of being efficiently handled and mechanically delivered to site. The reduction in seed volume and weight and the eventual use of artificial seed coats loaded with promoters, growth enhancing microbes (bacteria, mycorrhiza), and compounds that improve seed-soil interaction such as surfactants or wetting agents (Madsen et al. 2013), does provide opportunities for development of aerial seeding particularly in remote, difficult to access or vegetated landscapes.

### 4.5.4. *Next steps*

The main challenge is the upscaling of the sulphuric acid digestion process for large batch flow-through systems. Collaboration with engineers and seed producers is essential if such an innovation is to be used as a standardised tool in the native seed supply chain. Significant investment will be required to develop such equipment and procedures, but as the need for ecological restoration and consequent demand for native seed are expected to rise, the native seed industry should respond accordingly and advanced seed technology solutions will become an economic imperative.

## 4.6. Bibliography

Adkins SW, Simpson GM (1988) The physiological basis of seed dormancy in Avena fatua. IX. Characterization of two dormancy states. Physiol Plant 73:15–20 . doi: 10.1111/j.1399-3054.1988.tb09186.x

Bai ZG, Dent DL, Olsson L, Schaepman ME (2008) Proxy global assessment of land degradation. Soil Use Manag 24:223–234 . doi: 10.1111/j.1475-2743.2008.00169.x

Baskin CC, Baskin JM (2014) Seeds ecology, biogeography, and evolution of dormancy and germination, Second edi. Elsevier Ltd

Bewley JD, Bradford KJ, Hilhorst HWM, Nonogaki H (2013) Seeds. Springer New York, New York, NY

Broadhurst LM, Jones TA, Smith FS, et al (2016) Maximizing Seed Resources for Restoration in an Uncertain Future. Bioscience 66:73–79 . doi: 10.1093/biosci/biv155

Chambers JC, Macmahon JA (1994) A Day in the Life of a Seed: Movements and Fates of Seeds and Their Implications for Natural and Managed Systems Jeanne C. Chambers; James A. MacMahon Annual Review of Ecology and Systematics, Vol. 25. (1994), pp. 263-292. Ecology 25:263–292

Cole I, Metcalfe J, Koen T (2004) The effect of removing seed from florets on germination and field establishment in a Wallaby Grass (Austrodanthonia fulva) accession. Ecol Manag Restor 5:134–136 . doi: 10.1111/j.1442-8903.2004.201-3.x

Conant RT (2010) Challenges and opportunities for carbon sequestration in grassland systems. A technical report on grassland management and climate change mitigation. FAO

Davies C, Tallon S, Grey J, Miller F (1995) Characteristics of a Slot Flow Meter for Agricultural Seeds and Grains. In: 5th International Conference on Bulk Materials Storage, Handling and Transportation: Proceedings. Institution of Engineers, Barton ACT, pp 493–498

Erickson T, Barrett R, Merritt D, Dixon K (2016a) Pilbara Seed Atlas and Field Guide. CSIRO Publishing, Clayton South, Australia

Erickson TE, Muñoz-rojas M, Kildisheva OA, et al (2017) Benefits of adopting seed-based technologies for rehabilitation in the mining sector: a Pilbara perspective. Aust J Bot

Erickson TE, Shackelford N, Dixon KW, et al (2016b) Overcoming physiological dormancy in seeds of Triodia (Poaceae) to improve restoration in the arid zone. Restor Ecol 24:S64–S76 . doi: 10.1111/rec.12357

Ernst WHO, Veenendaal EM, Kebakile MM (1992) Possibilities for dispersal in annual and perennial grasses in a savanna in Botswana. Vegetatio 102:1–11 . doi: 10.1007/BF00031700

Fao (2014) Genebank Standards for Plant Genetic Resources for Food and Agriculture. Rev. ed. Rome.

Farley GJ, Bellairs SM, Adkins SW (2013) Germination of selected Australian native grass species, with potential for minesite rehabilitation. Aust J Bot 61:283–290 . doi: 10.1071/BT12258

Gang C, Zhou W, Chen Y, et al (2014) Quantitative assessment of the contributions of climate change and human activities on global grassland degradation. Environ Earth Sci 72:4273–4282 . doi: 10.1007/s12665-014-3322-6

Gibson-Roy P, Delpratt J, Campus B (2006) Seed resources for temperate native grassland restoration. Aust plant Conserv 15:2–3

Gibson-Roy P, Delpratt J, Moore G (2007) Restoring the Victorian Western (Basalt) Plains grassland. 1. Laboratory trials of viability and germination, and the implications for direct seeding. Ecol Manag Restor 8:114–122 . doi: 10.1111/j.1442-8903.2007.00348.x

Greipsson S (1999) Seed coating improves establishment of surface seeded Poa pratensis used in revegetation. Seed Sci Technol 27:1029–1032

Guan Y, Cui H, Ma W, et al (2014) An Enhanced Drought-Tolerant Method Using SA-Loaded PAMPS Polymer Materials Applied on Tobacco Pelleted Seeds. Sci World J 2014:9 . doi: 10.1155/2014/752658

Guzzomi AL, Erickson TE, Ling KY, et al (2016) Flash flaming effectively removes appendages and improves the seed coating potential of grass florets. Restor Ecol 24:S98–S105 . doi: 10.1111/rec.12386

Huarte R, Staltari S, Chorzempa SE, García MD (2007) Tripsacum dactyloides (L.) L. caryopses water uptake dynamics and germination responses to gibberellic acid, fluctuating temperatures and pericarp scarification. Seed Sci Technol 35:255–265 . doi: 10.15258/sst.2007.35.2.01

Humphreys AM, Antonelli A, Pirie MD, Linder HP (2011) Ecology and evolution of the diaspore "burial syndrome." Evolution (N Y) 65:1163–1180 . doi: 10.1111/j.1558-5646.2010.01184.x

James JJ, Svejcar TJ, Rinella MJ (2011) Demographic processes limiting seedling recruitment in arid grassland restoration. J Appl Ecol 48:961–969 . doi: 10.1111/j.1365-2664.2011.02009.x

Larson JE, Sheley RL, Hardegree SP, et al (2015) Seed and seedling traits affecting critical life stage transitions and recruitment outcomes in dryland grasses. J Appl Ecol 52:199–209 . doi: 10.1111/1365-2664.12350

Lewandrowski W, Erickson TE, Dixon KW, Stevens JC (2017) Increasing the germination envelope under water stress improves seedling emergence in two dominant grass species across different pulse rainfall events. J Appl Ecol 54:997–1007 . doi: 10.1111/1365-2664.12816

Ling, Guzzomi, Erickson, et al (2015) Seed Ablation

Loch DS, Johnston PW, Jensen TA, Harvey GL (1996) Harvesting, processing, and marketing Australian native grass seeds. New Zeal J Agric Res 39:591–599 . doi: 10.1080/00288233.1996.9513218

Ma HY, Liang ZW, Wang ZC, et al (2008) Lemmas and endosperms significantly inhibited germination of Leymus chinensis (Trin.) Tzvel. (Poaceae). J Arid Environ 72:573–578 . doi: 10.1016/j.jaridenv.2007.06.013

Madsen MD, Kostka SJ, Hulet A, et al (2013) Surfactant Seed Coating – a Strategy To Improve Turfgrass Establishment on Water Repellent Soils. In: International Symposium on Adjuvants for Agrochemicals. pp 205–210

McDonald T, Jonson J, Dixon KW (2016) National standards for the practice of ecological restoration in Australia. Restor Ecol 24:S4–S32 . doi: 10.1111/rec.12359

Merritt DJ, Dixon KW (2011) Restoration Seed Banks--A Matter of Scale. Science (80- ) 332:424–425 . doi: 10.1126/science.1203083

Mitchley J, Jongepierová I, Fajmon K (2012) Regional seed mixtures for the re-creation of species-rich meadows in the White Carpathian Mountains: results of a 10-yr experiment. Appl Veg Sci 15:253–263 . doi: 10.1111/j.1654-109X.2012.01183.x

O'Mara FP (2012) The role of grasslands in food security and climate change. Ann Bot 110:1263–1270 . doi: 10.1093/aob/mcs209

Parish RL, Bracy RP (2004) Recommendations for Effective Use of a Garden Seeder for Research Plots and Gardens. Horttechnology 14:257–261

Pedrini S, Merritt DJ, Stevens J, Dixon K (2017) Seed Coating: Science or Marketing Spin? Trends Plant Sci 22:106–116 . doi: 10.1016/j.tplants.2016.11.002

R Core Team (2015) R: A Language and Environment for Statistical Computing.itle

Ritz C, Baty F, Streibig JC, Gerhard D (2015) Dose-response analysis using R. PLoS One 10:1–13 . doi: 10.1371/journal.pone.0146021

Ritz C, Streibig JC, Ritz, C. & Streibig JC (2005) Bioassay Analysis using R. J Stat Softw 12:1–22 . doi: 10.18637/jss.v012.i05

Society for Ecological Restoration International Science & Policy Working Group (2004) The SER International primer on ecological restoration. Tucson

Stevens J, Chivers I, Symons D, Dixon K (2015) Acid-digestion improves native grass seed handling and germination. Seed Sci Technol 43:313–317 . doi: http://dx.doi.org/10.15258/sst.2015.43.2.19

Vaughn KJ, Young TP (2015) Short-term priority over exotic annuals increases the initial density and longer-term cover of native perennial grasses. Ecol Appl 25:791–799 . doi: 10.1890/14-0922.1.sm

Waters C, Whalley W, Huxtable C (2017) Microlaena stipoides (Microlaena or Weeping grass). In: Grassed up - Guidel. Reveg. with Aust. Nativ. grasses. http://www.dpi.nsw.gov.au/agriculture/pastures-and-rangelands/rangelands/publications-and-information/grassedup/species/microlaena

Whalley RDB, Jones CE (1996) Commercialisation of the Australian Native Grass Microlaena Stipoides for Turf, Amenity and Forage Purposes. University of New England, Department of Botany

White R, Murray S, Rohweder M (2000) Pilot Analysis of Global Ecosystems: Grassland Ecosystems. Washington D.C.

# 5. Seed encrusting with salicylic acid: a novel approach to improve surival of native grasses

## 5.1. Introduction

Nearly two-thirds of the world ecosystems are considered degraded (Nellemann and Corcoran 2010). Such degradation poses a serious risk to biodiversity and impacts human communities that rely on ecosystem services for their sustenance and wellbeing (Costanza et al. 1997; Palmer and Filoso 2009). Once degradation has occurred, restorative activities are needed to return the functionality, diversity, and structure of healthy, intact, and sustainable ecosystems (Society for Ecological Restoration International Science & Policy Working Group 2004; McDonald et al. 2016). Grasslands, extending over 52.5 million square kilometres globally, are among the largest terrestrial ecosystem in the world (Suttie et al. 2005) and provide some fundamental services such as: sustaining food production (meat and milk), carbon sequestration and storage (O'Mara 2012), and erosion control. However, according to Gang et al. (2014), almost 50% of grassland worldwide is degraded due to human activities and climate change.

When the level of disturbance is too severe, spontaneous regeneration may not be feasible or effective, and restorative measures may be required (Gibson 2009). Native seeds of appropriate-local origin are commonly used to reintroduce missing species and to perform ecosystem reconstruction when the land has been completely cleared (Conrad and Tischew 2011), like in post-mining or post farming. Such scenarios pose numerous challenges to restoration practitioners. Abiotic factors such as poor, chemically or physically adverse soil (Cross et al. 2017) and low or unpredictable water availability (Lewandrowski et al. 2017) combined with biotic variables such as seed predation (Orrock et al. 2008) and competition with exotic species could limit the success of seed-based grassland restoration.

Generally, less than 10% of sown seed ultimately grow into established plants, with the major bottlenecks detected at the seedling emergence phase (James et al. 2011), and survival through the drought cycles of the first summer (Pyke 1990). Given the high cost and low availability of native seed (Merritt and Dixon 2014), improving the efficiency in their use is crucial, if ecological restoration has to be delivered at the landscape scale that the current rate of degradation requires (Menz et al. 2013). To

address issues related to the logistical aspect of seed delivery and seed performances, the crop seed industry has developed technologies, such as seed coating, that could be adapted and applied to native seed (Pedrini et al. 2017). Seed coating is the practice of covering seeds with external materials, loaded with active ingredients that provide protection and enhance seed performances (Taylor et al. 1998). Seed coating is usually categorised on the level of physical modification of the seed. Seed encrusting is an intermediate kind of coating, where seed volume and weight are increased, but the shape of the original seed is still evident. Seed coating was tested on native seeds in different restoration scenarios to overcome specific limitation such as water repellency (Madsen et al. 2013), soil crusting (Madsen et al. 2012), and seed predation (Pearson et al. 2018). Despite promising results in improving seedling emergence, limited studies have so far attempted to improve seed germination and seedling resistance to abiotic stresses.

Such resistance could be provided by Salicylic Acid (SA). SA is a plant hormone, synthesised at different degrees by all plant species (Raskin et al. 1990). It is involved in plant growth, development regulation (Rivas-San Vicente and Plasencia 2011), signalling (Park et al. 2007), thermogenesis and mediating stress response either by providing resistance or triggering apoptosis (Dat et al. 2007). Exogenous application of SA through watering, foliar spray, or seed imbibition has shown increased plant resistance and survival to a wide range of abiotic (Horvat et al. 2007) and biotic stresses (Durner et al. 1997). SA efficacy in delivering stress resistance is a function of its concentration, with low concentration failing to deliver resistance and higher concentration decreasing resistance by activating cell death pathways (Senaratna et al. 2000; Stevens et al. 2006). SA effect on seed germination is not yet clear. Some studies reported improvement in germination for *Arabidopsis thaliana* under salinity (Lee and Park 2010) and for wheat (*Triticum aestivum)* under drought stress (Sharafizad et al. 2013), whilst no effect was detected in maize (*Zea mays*) (Xie et al. 2007) and barley (*Hordeum vulgare)* (Guan and Scandalios 1995). Seed coating delivery of SA has shown some promising results when tested on tobacco seed, improving seed germination and seedling growth under drought stress (Guan et al. 2014), and on corn, inducing resistance to chilling tolerance (Guan et al. 2015). However it has never been tested on native species for ecological restoration.

This study aimed to evaluate the effects of salicylic acid and treatments for SA delivery (imbibing and encrusting), on seed germination, seedling emergence, seedling survival

and seedling growth for three native grass species. The following hypotheses were tested:

- The encrusting and imbibing processes will not detrimentally affect seed germination or seedling emergence compared with untreated seeds.
- Salicylic acid will improve germination under water stress and enhance seed germination and seedling emergence in the field
- Survival and growth in the field will be improved in plant established from SA treated seeds.

When a significant improvement is detected, the SA delivery methods (imbibing and encrusting) are compared to determine which treatment is more effective in delivering SA.

## 5.2. Material and methods

### 5.2.1. *Seed selection and cleaning*

The seeds of three grass species native to the southern-temperate part of Australia were selected for their extensive use in grassland revegetation/restoration and pasture (Waters et al. 2001): *Austrostipa scabra* (Lindl.) S.W.L.Jacobs & J.Everett, *Microlaena stipoides* (Labill.) R.Br. var. Griffin and *Rytidosperma geniculatum* (J.M.Black) Connor & Edgar var. Oxley. Seeds were provided by the Native Seed PTY Ltd (Cheltenham, Victoria) in 2016. To decrease viability loss due to inappropriate storing, seeds were stored in paper bags on open shelving at 15° C, and 15% relative humidity (RH) controlled environment for one year. Seeds were moved to ambient condition at 20°-25° C and 40-50% RH two weeks prior treatment, to allow for a period of acclimatisation, and avoid potential seed damage during the processing and encrusting process.

Before encrusting and imbibition, the caryopses for each species were extracted from the husk to allow for more homogeneous treatment. Removal of the palea and lemma was performed for each species using sulphuric acid digestion following the methodology originally described by Stevens et al. (2015). This treatment consists of the complete immersion of the florets in a Sulphuric acid solution (ACS reagent grade $H_2SO_4$, Sigma-Aldrich, St Louis, USA) for enough time to allow for the weakening of the floret structures, without reducing the germination performances. Immersion time for

this species was tested and determined by Pedrini et al. (2018) and consist of 90 minutes for *A. scabra*, 60 minutes for *M. stipoides* and 20 minutes for *R. geniculatum*. The acid immersion was then followed by a neutralisation treatment in a 8.4 g $L^{-1}$ sodium bicarbonate ($NaHCO_3$, Sigma-Aldrich, St Louis, USA) solution for 5 minutes, two minute rinsing under tap water and concluded with a three hour drying process in a Food Lab™ Electronic Dehydrator at 35° C (Sunbeam, Sydney, Australia).

In each species, the acid digestion was not enough to completely remove the palea and lemma, but the weakening of the external floret components allowed for a rapid caryopsis extraction through gentle rubbing with a rubber mat, and subsequent use of sieves and a zig-zag air flow separator (Selecta Machinefabriek BV, Enkhuizen, Netherlands).

### 5.2.2. *Seed treatments*

After cleaning, the caryopses of each species were subjected to either seed imbibition or encrusting processes (Figure 5.1). For each process, treatment with or without Salicylic Acid was applied, ultimately resulting in four treatments plus one untreated control: Imbibed without SA, imbibed with SA, encrusted without SA, encrusted with SA and control.

SA was provided at a concentration of 0.1 mM, which is within the range of effective induced stress resistance across various species and delivery methods tested (Stevens et al. 2006; Janda et al. 2007; Khan et al. 2015). SA solution was prepared by dissolving crystalline SA (Sigma Aldrich, St. Louis, USA) in deionized water for imbibition and in a 2% Hydroxyethyl cellulose hydroxyethyl cellulose (cellosize QP 09-L, DOW chemicals) solution for encrusting, and mixed with a magnetic stirrer for 30 minutes at 50° C. For the imbibition treatments, seeds were soaked in either SA solution or deionized water for 24 h at 20 °C in the dark.

Seed encrusting was performed on a 15 cm RRC 150 Lab Coater (Centor Thai, Bangkok, Thailand), following the protocol described by Pedrini et al. (2018). The liquid was delivered through a compressed air propelled 0.7 mm airbrush (Ozito tools, Australia). Talc was used as filler material and was dusted onto the seed with a paint brush. 10 g of cleaned seed were placed inside rotary coater, and the rotor speed set at 300 RPM. Seeds were initially exposed to liquid spray until moist and then the powder was dusted on the rotating seed mass. Wetting and dusting were repeated until 20 g of

powder was used. A total of 15 ml of liquid was applied. During the process, the seeds were routinely checked to visually evaluate the even coverage of the coat and to assess the formation of multiple seeds or dead balls (agglomerate of coating material without a seed inside).

At the end of the imbibition and encrusting process, seeds were placed on trays and dried for 3 hours in a forced air dehydrator at 35°.

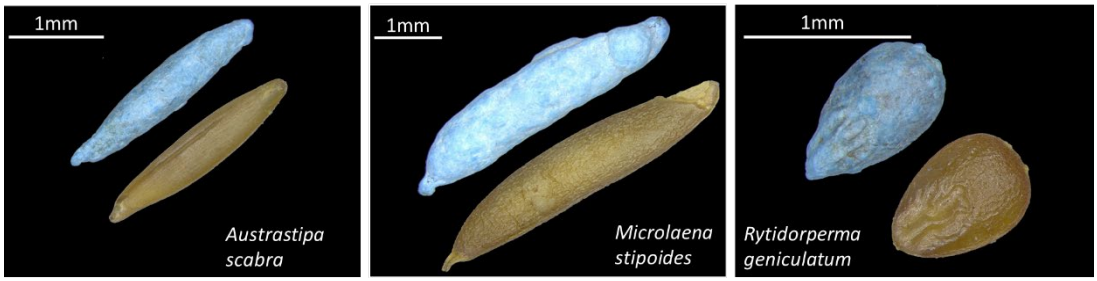



*Figure 5.1: Seeds of the three grass species tested. In each image are presented the encrusted (blue) and untreated-imbibed seed. The images of the species have been taken at different magnification. Each image present its scale for reference*

### 5.2.3. *Laboratory test*

Germination test was performed in Petri dishes lined with two filter papers moistened with 14 ml water or Polyethylene Glycol (PEG) solution, placed in sealed plastic bags to reduce dissection. 2 ml of water or PEG solution was added weekly.

PEG 8000 (Sigma-Aldrich, St Louis, USA) diluted in deionised water at 24.72, 30.78, and 35.90 g/l to was used to obtain solutions of -0.6, -0.9, and -1.2 MPa water potential at 20° C. This value resembles the range of water availability recorded in the field (Pedrini unpublished). The test was performed on 25 seed per Petri dishes, 4 replicates per treatment. Petri dishes were located in a Biosyn incubator 6000 OP (Contherm, Korokoro, New Zealand) at 20°C with a 12 h photoperiod.

Germination was scored daily for the first five days and then at 7, 10 and 15 days respectively. On the 21st day, final germination was scored and remaining seed examined via cut test to assess viability. Non-viable seeds were excluded from the total.

### 5.2.4. *Field trials*

Field trials were performed on a site in the hills east of the town of Waroona (Western Australia), located at 32°74'27" S, 116°00'36" E, at 201 m altitude. The site was

selected for being within the native range of the three tested species, and closely resembling the climatic conditions of mining operations active in the area that will require seed based restoration after mine closure. The field trial area was enclosed by a fence to avoid grazing from native marsupials and rabbits. Three experiments were performed in the field site: germination in bags, emergence in line, and emergence in plots. The five treatment previously described were tested in each experiment. For germination experiment, each treatment had four replicates, randomly distributed. Lines and plots were arranged on a randomised complete block design of four blocks for 15 treatments (5 treatments * 3 species)

#### *5.2.4.1. Germination bags experiment*

Field seed germination was tested by placing 50 seeds in 5 $cm^2$ sealed mesh bags, over a 2 $m^2$ area, and buried on site at 1mm depth. The bags were collected three weeks after sowing and germination recorded for those seeds where a protruding radicle was detected.

#### *5.2.4.2. Line experiment*

Seedling emergence was tested sowing 100 seeds along metre-long lines. Seeds were sown on the 9th of May 2017. Seedling emergence was scored after 1, 2, 3, 4, 6, 8 and 10 weeks. Further scoring after 45 weeks was performed to record plant survival over the summer season.

#### *5.2.4.3. Plot experiment*

To evaluate plant survival and growth, 100 seeds were manually broadcasted on a 0.5 x 0.5 $m^2$ plot. A month after sowing, the plots were thinned to 10 randomly selected seedlings, with at least 5 cm buffer between seedlings to limit potential competition. The selected seedlings were marked with a pin to avoid confusion with other seedlings that could have emerged at a later stage. 45 weeks after sowing the surviving plants were counted, harvested and their height, wet weight and dry weight recorded.

Sowing depth between 0.2 and 0.5 cm was achieved by broadcasting dry soil on top of freshly sowed lines and plots.

Soil temperature and volumetric moisture content ($m^3/m^3$) were recorded for the duration of the germination and emergence experiment (10 weeks) with HOBO Micro

Station Data Loggers (Onset Computer Corporation, Bourne, MA, USA). The probes were buried at 1 cm. In the 35 weeks following the end of the emergence experiment, until survival and growth were recorded (July 2017 – March 2018), minimum and maximum temperature and precipitation data were obtained from the Dwellingup weather station, 10 km from the site (BOM 2018) (Figure 5.2).

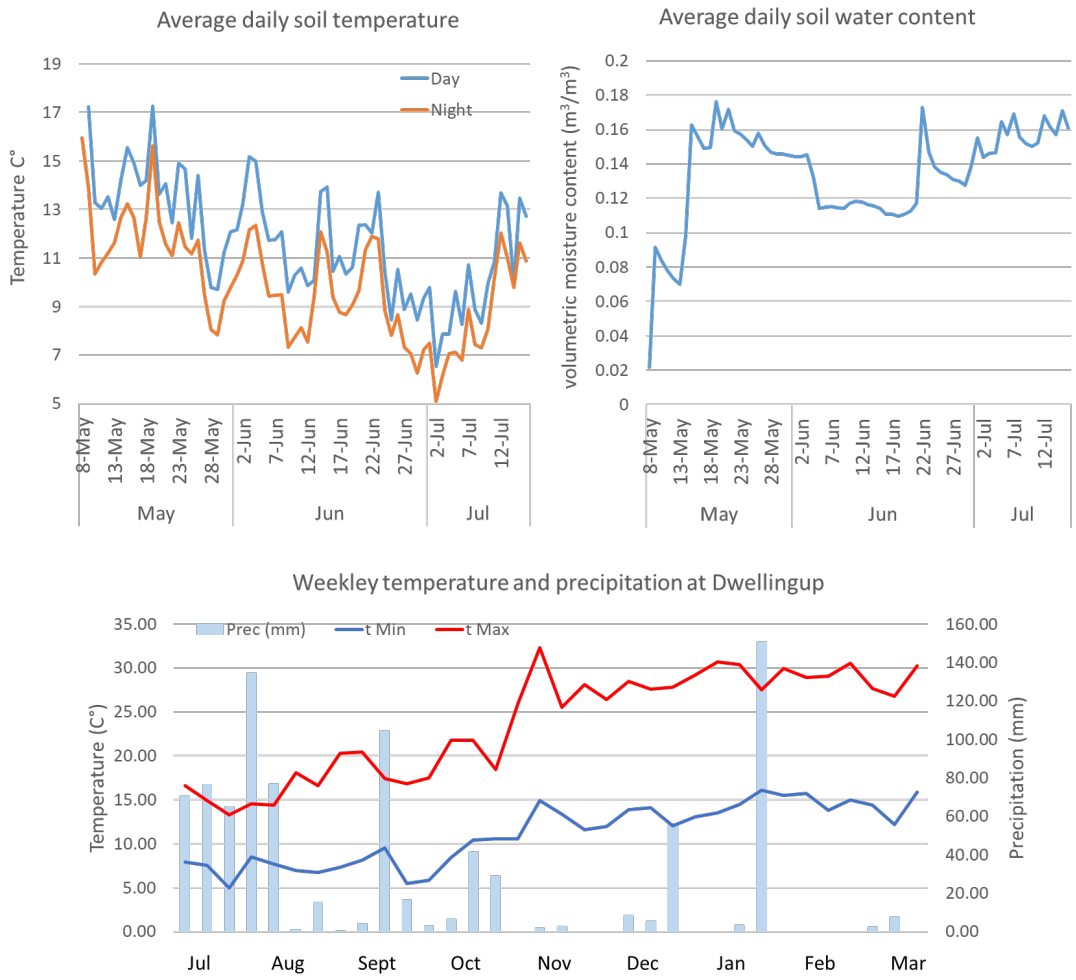



*Figure 5.2: Field site climate conditions. The tables on the first row present the daily average for day (orange) and night (blue) temperature and volumetric water content in the soil at 1 cm depth for the first 10 weeks of the experiment, while germination and emergence were recorded. The table on the second row presents the weekly maximum (red line) and minimum (blue line) temperature, and total precipitation (blue bars) for the period between the end of the emergence experiment and the recording of plant survival (July 2017 – March 2018). Data were obtained from the weather station located at Dwellingup (Western Australia) 10 km from the field site.*

### 5.2.5. *Statistical analysis*

Data analysis was performed on the software R (R Core Team 2015). To assess laboratory germination and seedling emergence in the field, non-linear regression

models were fitted with the function “drm” of the “DRC” package (Ritz et al. 2005, 2015; Lewandrowski et al. 2017). A three parameter log-logistic model was used:

$$f(x) = \frac{gmax}{1 + \left(\frac{x}{T50}\right)^{b}}$$

The parameters are: (b) slope curvature, (gmax) final germination and (T50) germination speed, intended as time (days/weeks) required to reach half of the final germination or emergence. Parameter comparison on final germination and germination speed were then performed to assess differences among treatment (significance $p < 0.05$).

To test the hypothesis of treatment and compound effect on germination in the field (in buried bags) and plant survival, an exact binomial test on the probability of success in a Bernoulli trial, between each treatment, was performed (confidence level = 0.95).

Plant height and biomass data were fitted in a Linear Mixed-Effects Model using the “lmer” function in the lme4 package for R (Bates et al. 2015), using compounds (ctrl vs SA vs NO) and treatment (Ctrl, Imb and Encr) as fixed variables and the replicates (plots) as a random variable.

Anova (Type II Wald chi-square tests) was used to detect a significant treatment effect. If such significance was detected a pairwise t-test was performed to compare the levels within the treatment.

## 5.3. Results

In the first two sections the results of seed germination in a controlled laboratory setting and seed germination/emergence in field experiment are presented, while in the third section plant survival data, collected at the field site, are examined along with height and weight of plant harvested at the end of the experiment. In each section, the results will be presented by species, comparing laboratory and field experiment. This approach could be more helpful in understanding treatment effect, than comparing the result of a single experiment across different species.

### 5.3.1. *Encrusting and imbibition treatment*

Encrusting treatment (Encr) had higher or similar germination than the control (Ctrl), whilst imbibition treatment (Imb) at times resulted in lower germination. For *A. scabra,* germination of treated seed in a controlled environment did not show any significant difference in final germination, and only a slight but significant ($P < 0.001$) increase in germination speed (T50) of 0.5 days, for both imbibed and encrusted seed. When tested in field conditions, the encrusted seed had lower final emergence than the control (Ctrl: 52 ± 1.6%, Encr: 45 ± 2.4%, $P < 0.001$) while imbibed seeds showed no significant difference (Figure 5.3).

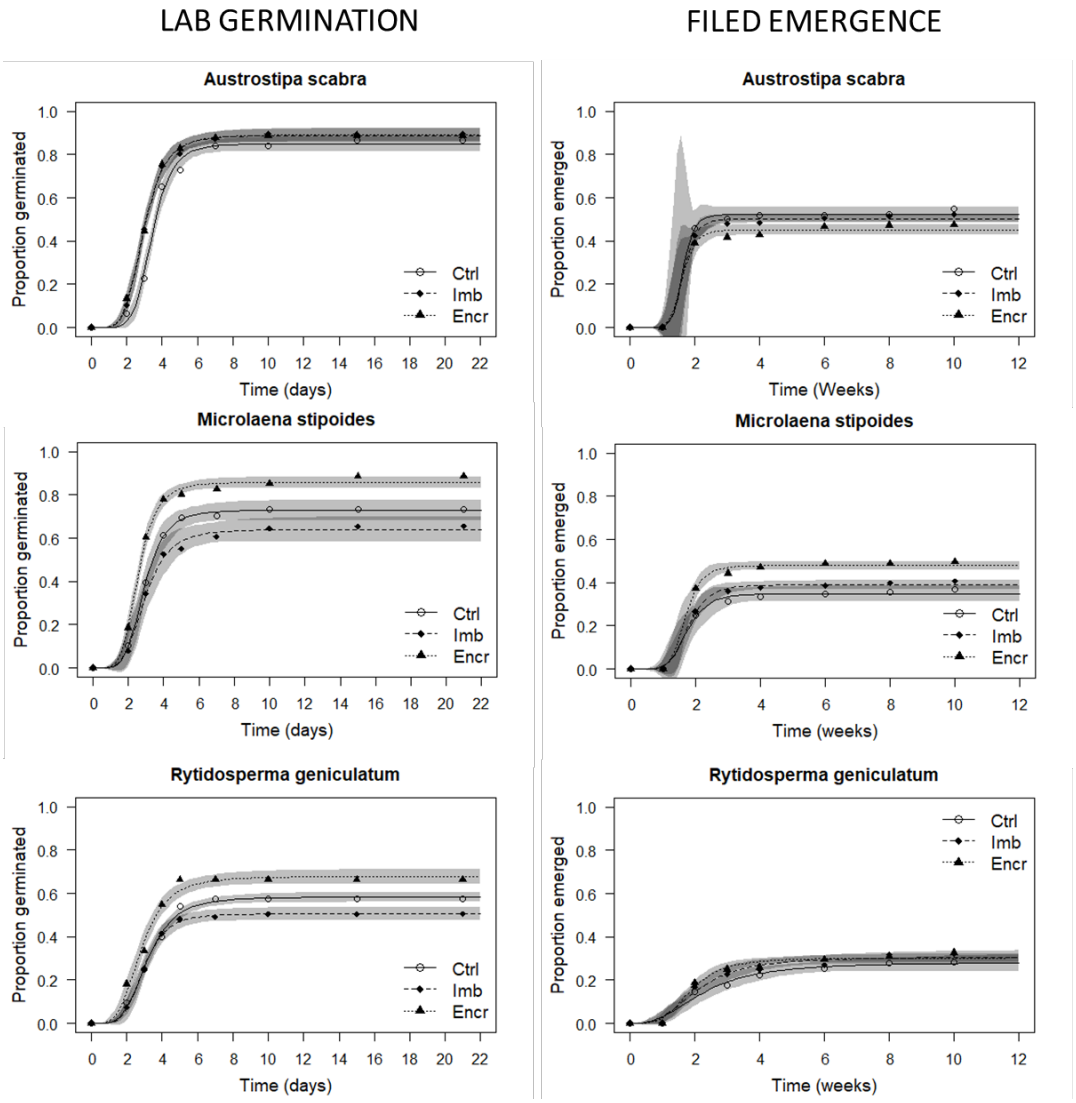



*Figure 5.3: Cumulative germination/emergence percentage curve of the three different seed treatment tested: untreated (ctrl), encrusted (Encr), and imbibed (Imb) across the three species tested. The lines represent the cumulative germination curve over time. The dots show the germination recorded on a specific day/week and the shaded area around the germination curves represent the 95% confidence intervals. The graphs in the first*

*line represent the germination experiment in controlled laboratory condition. The graph in the second line shows the results of seedling emergence in field trial.*

In controlled laboratory setting, encrusted *M. stipoides* seeds germinated more than the control (Encr: 86 ± 2.1%, Ctrl: 73 ± 2.2%, $P < 0.001$), while germination was 8.9% lower for imbibed seed ($P < 0.05$). Similarly, final emergence in the field was higher for encrusted seed (Encr: 48 ± 1.0%, Ctrl: 35 ± 1.0%) and, differently to what was shown by the germination test, imbibition improved germination by 4% ($P < 0.05$).

Like *M. stipoides,* germination of *R. geniculatum* was significantly higher in encrusted seeds (68 ± 1.5%) and worst for imbibed seeds (51 ± 1.4%), (Ctrl: 58 ± 1.5%). However, there was no difference in emergence in response to seed treatment.

### 5.3.2. *Salicylic acid effects on germination with low water availability and field emergence*

To assess the effect of Salicylic acid (SA), seeds that were provided SA (via imbibition and encrusting) were compared to seeds that have received the treatments without SA (NO). If a significant difference was detected, SA delivery methods of encrusting (ES) and imbibing (IS) were then compared. The high variability in the results suggested that SA has limited effects on promoting germiation and emergence.

Final germination at optimal water potential in *A. scabra* was significantly ($P < 0.05$) reduced by 4.3% with SA treatment, but no difference was detected in final germination for seed with SA delivered via imbibing or coating (Figure 5.4). At reduced water availability of -0.6, -0.9, and -1.2 MPa SA treatments generally showed a slight improvement in final germination, but never significant. When tested in the field SA treatments didn't affect germination but reduced final emergence (NO: 51 ± 1.1%, SA: 44 ± 1.1%, $P < 0.001$). SA encrusted seed emerged 5.6% less than SA imbibed seeds.

Similarly, *M. stipoides* germination at optimal conditions was reduced in SA treated seed by 7.9% ($P<0.05$). SA delivered through encrusting resulted in better germination (77 ± 2.1%) than SA imbibed seed (57 ± 2.2%). On simulated water stress scenario at -0.6 MPa, germination for SA treated seed was improved from 77% ± 1.9% to 86 ± 1.9%, and encrusting allowed for a 12.7% increase in germination compared to imbibing. However, at lower water potential SA treatment reduced final germination by 5.6% ($P < 0.05$) at -0.9 MPa and by 11.2% ($P < 0.01$) at -1.2MPa. In both situation encrusting allowed for better germination then imbibition. Field germination and emergence of *M.*

*stipoides* were not significantly affected by SA treatment, but both treatments had higher emergence than the untreated control.

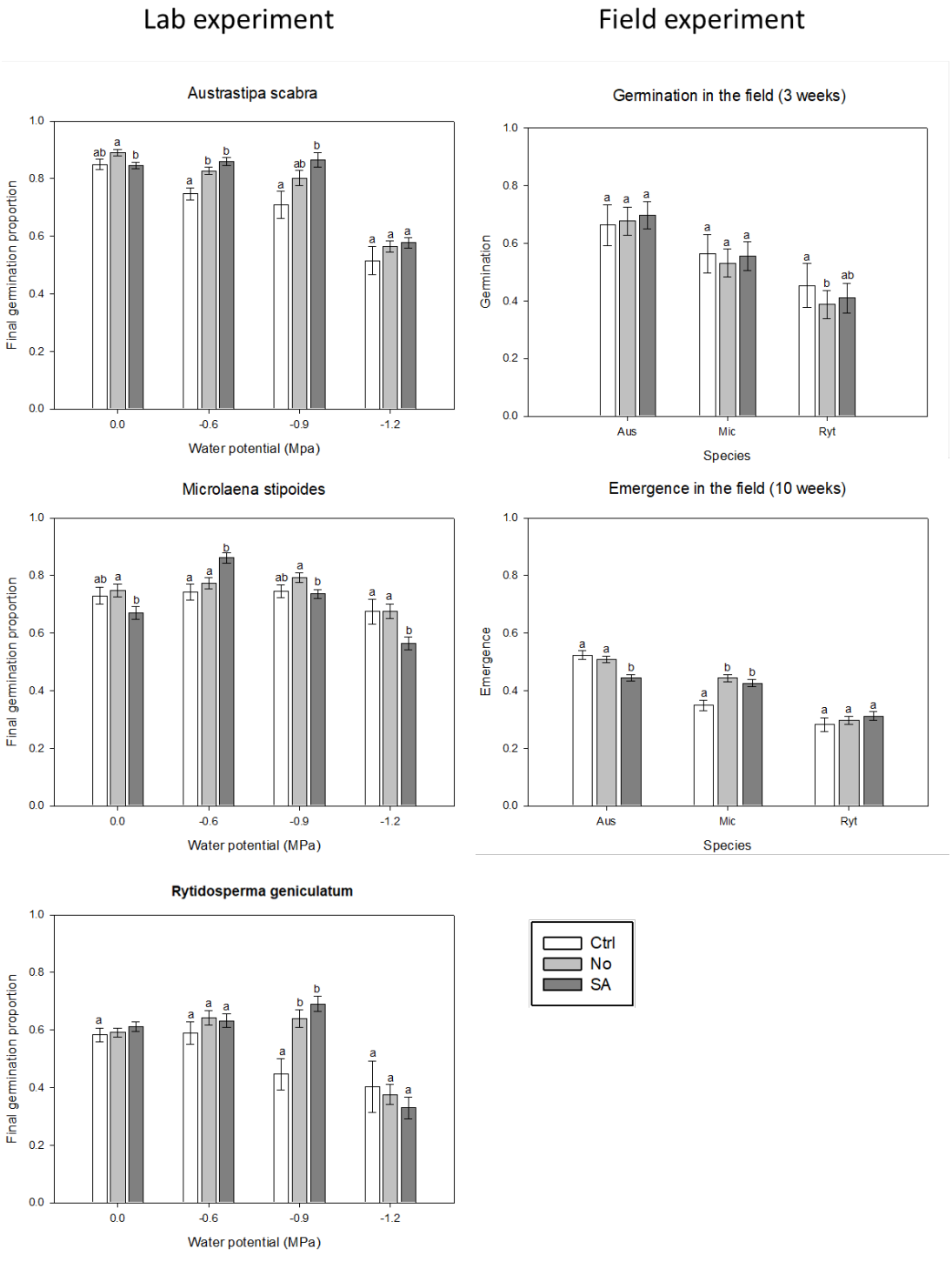



*Figure 5.4: Final germination and emergence proportion of untreated used (Ctrl), seed treated without Salicylic Acid (No) and seed treated with Salicylic acid (SA). In the top row are presented the graphs relative to the laboratory germination experiment in petri dishes at 20°c at different water potential (X axis). In the bottom row are shown the germination and emergence results in the field experiment, respectively 3 and 10 weeks after sowing. The number next to the treatment code is the exposure time in minutes. The species are listed in the X axis (Aus = Austrastipa scabra, Mic = Microlaena stipoides, Ryt = Rytidosperma geniculatum). Results followed*

*by the same letter for the Water potential (lab experiment) and species (Field experiment) are not statistically different at p < 0.05*

When final germination was tested on *R. geniculatum,* no significant difference between seed treated with and without SA was detected at optimal conditions and with reduced water availability. The only effect of SA was a delay in germination at 0.0MPa of 0.4 days. Field germination was not different for seed treated with and without SA however, both treatments had lower germination than the untreated control. Between seeds treated with and without SA, there was no difference in field germination. However, seed treated without SA had significantly lower germination ($p<0.05$) than the untreated control. Emergence in SA treated seeds was slightly higher, but not significant.

### 5.3.3. *Survival and plant growth in field site conditions*

Plant survival was examined in situations where interspecific competition was reduced (plot experiment) and where emerged seedling were not thinned (line experiment). In both scenarios, SA improved plant survival and growth.

In reduced competition (plot) experiment, the average survival of untreated control was of 82.5% for *A. scabra*, 82.5% for *M. stipoides* and 77.5% for *R. geniculatum*. In *A. scabra*, survival was improved by 6.25% for SA treatments compared to seed treated without SA, but the difference was not significant. SA treated *M. stipoides* and *R. geniculatum*, survival was significantly improved ($P<0.01$), by 8.2% and 15% respectively. SA delivered through encrusting provided slightly better survival that imbibed, but not significant. Both for *M. stipoides* and *R. geniculatum,* SA treatment improve survival by 17.5% and 10% respectively, compared to the untreated control (Figure 5.5).

In the lines experiment, where all emerged seedling were maintained, the survival of plant that emerged from untreated seed was of 32.3% for *A. scabra*, 41.2% for *M. stipoides* and 42.6% for *R. geniculatum*. Plants emerged from SA treated seed, compared to seeds treated without SA, had a significantly ($P>0.001$) increased survival of 12.9% in *A. scabra*, 13.5% in *M. stipoides* and 11.8% in *R. geniculatum*. In *A. scabra*, SA delivered through encrusting improve survival by 9.8% ($P >0.001$) compared to SA delivered through imbibing. In *M. stipoides* and *R. geniculatum*, no difference was detected between SA delivery systems on plant survival.

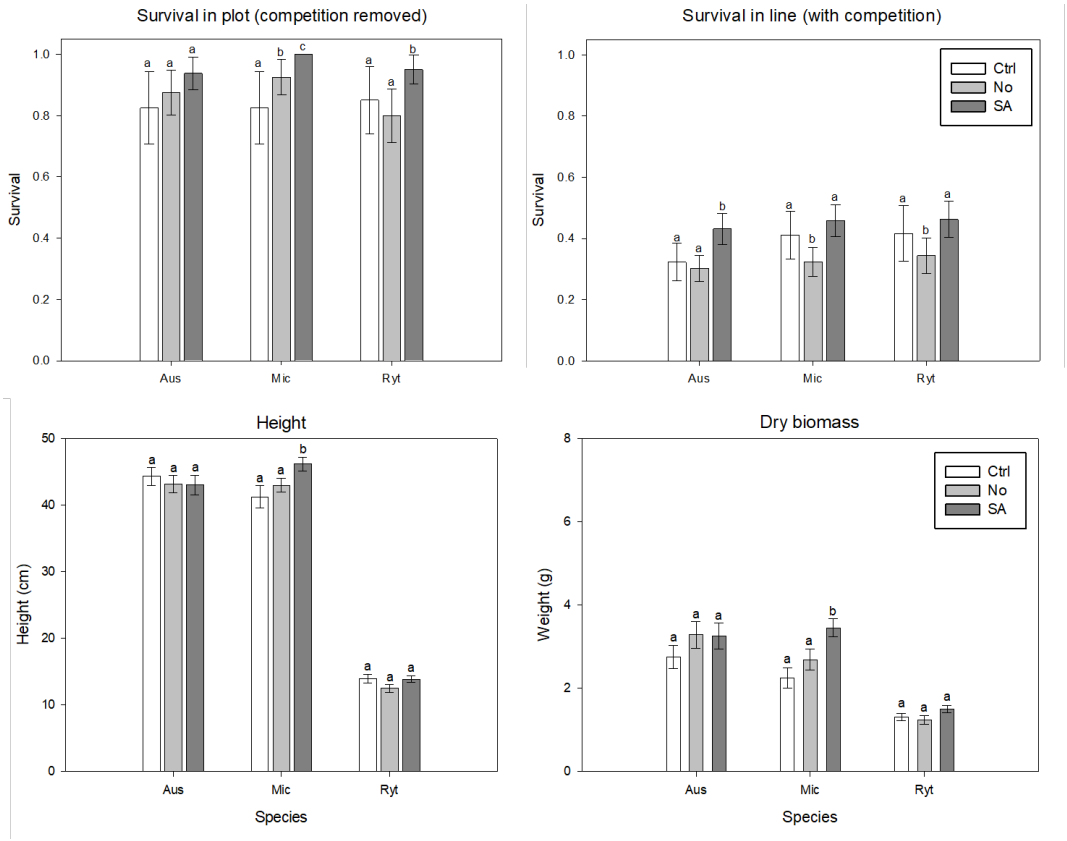



*Figure 5.5: Survival and plant growth comparison 40 weeks after sowing, between untreated used (Ctrl), seed treated without Salicylic Acid (No) and seed treated with Salicylic acid (SA). In the top row are presented survival data. The graph on the left shows plant survival proportion in the plot experiment, where interspecific competition was limited, by removing excess seedling and leaving 10 seedlings per 0.25 m $^2$ plot. For "survival in line" graph, on the top right, seeds were sown on a 1 m line and seedlings were not removed after emergence. In the bottom row average height (left) and biomass (right) of plant collected from the plot experiment. Results followed by the same letter for the Water potential (lab experiment) and species (Field experiment) are not statistically different at p < 0.05.*

Plant growth was recorded in term of plant height and above ground dry biomass. In *A. scabra*, no significant difference was detected between SA and NO treatments in either measurement. For *M. stipoides*, plant height for SA treated seed was significantly improved (P<0.05) from 41 cm ± 1.7 cm (untreated control) and 43 cm ± 1.0cm (treated seed without SA), to 46 cm ± 1.0 cm. Dry above-ground biomass was also higher in SA treatment (3.4 g ± 0.22g) compared to untreated control (2.2 g ± 0.25 g) and treated without SA (2.7 g ± 0.25 g) (both P<0.05). In *R. geniculatum*, there was no significant difference in height. Dry biomass of SA treated seed (1.5 g ± 0.08g) was significantly higher (P>0.05) than treated without SA (1.2 g ± 0.10g), but not significant compared to the untreated control (1.3 g ± 0.09g). A significant difference between SA delivery through imbibing or encrusting, in term of plant growth, was detected in none of the species.

## 5.4. Discussion

### 5.4.1. *Seed treatment effects on germination and emergence*

Of the three species tested, only *A. scabra* showed no treatment (encrusting and imbibition) effect on germination and emergence as hypothesised. *M. stipoides* and *R. geniculatum* showed unexpected, significant differences between treated seeds and the control. In the germination experiment, the two species behaved similarly, with encrusted seeds performing better than controls, while imbibition had negative effects on both final germination and germination speed. In this study, seeds were imbibed for 24 hours, following methodology previously tested for SA delivery to seeds (Senaratna et al. 2003; Sharafizad et al. 2013). A potential explanation for the reduction in germination of imbibed seed could be anoxic stress due to extended submersion in water and in a water-saturated environment (petri dish). This problem has been reported in seed priming treatments that rely on seed imbibition to trigger pregermiantive metabolic mechanism (Chojnowski et al. 1997). Low oxygen availability could also explain why encrusted seed performed better than imbibed and untreated seed. During the encrusting process, seed contact with water was limited compared to imbibing. Moreover, the layer of encrusting material could also have acted as a buffer, reducing the water potential at the seed level and allowing for better gas exchanges. Furthermore, the emergence of imbibed seed was unaffected in the moist, but not water-saturated soil conditions. In seed priming treatments, water potential or water oxygenation are usually regulated (Bujalski and Nienow 1991) to avoid anoxic damage. The germination reduction detected in this study for imbibed seed could, therefore, be mitigated by decreasing imbibition time, reducing the water potential, or providing oxygenation to the solution.

### 5.4.2. *Salicylic acid effect on seed germination and emergence*

Contrary to what was initially hypothesised, SA application did not clearly improve seed germination and emergence in the field and in controlled lab condition across a water availability gradient on the tested species, with the exception of *M. stipoides* at -0.6 MPa. *M. stipoides* seed treated with SA had significantly lower germination at 0.0, -0.9 and -1.2 MPa, suggesting that this species might be susceptible to the SA concentration tested. Germination response to exogenous SA application is concentration dependent, with inhibition detected at higher concentrations (Guan and

Scandalios 1995). Reducing SA concentration for *M. stipoides*, could therefore potentially remove the germination impediments. When a difference in germination was detected for seed treated with SA, encrusted seed performed slightly better than imbibed. However, this difference is most likely due to the processing itself, as highlighted previously, other than the efficacy in delivering SA.

A significant drop in emergence by SA treated seed in *A. scabra*, in field emergence experiment, might suggest that the interaction of SA treatment with unidentified variables present in the soil at field site might have triggered a negative response, similar to what was observed in the controlled lab environment. Moreover, the detrimental effect of encrusting could have been determined by the combined effect of SA and the physical constraint of the coatings layer and soil to the emerging seedling. However, this effect was not detected in the other species.

### 5.4.3. *Survival and growth*

In experimental plots where competition was removed, plants from seed treated with SA resulted in increased height and biomass production in two out of the three species tests. SA also provided a significant improvement in plant survival in both the scenarios with interspecific competition maintained and removed. Although response among species was varied, with the least effects detected in *A. scabra*, the overall trend showed marked benefits in term of survival and plant grown from SA-treated seeds. The improved survival at this stage could be explained by the already described stress resistance proprieties of SA (Khan et al. 2015).

#### *5.4.3.1. Demographic processes*

In field experiments, soil conditions at the time of germination and emergence (Figure 5.2) were suitable for the germination of these temperate grass species. Differently to what was described by James et al. (2011), where the major bottleneck in seedling recruitment was detected at the emergence phase (when germinated seeds failed to push through the soil), in this experiment, the drop between germination and emergence was relatively small with probability of emergence from germinated seed ranging from 0.92 in *A. scabra* to 0.61 in *R. geniculatum* (Figure 5.6). This trend might be due to the favourable climatic and soil conditions during the year the study was conducted, with average night and daily temperature ranging between 10° C and 18° C, and maintained soil moisture content of 0.08-0.18$m^3/m^3$ (water potential range

between -0.2 and -0.7 Mpa) during the first month after sowing, when most of the emergence has occurred. These conditions have not allowed for the detection of stress resistance inducing proprieties of SA that were originally hypothesis at the germination and emergence phase. However, the field data, combined with the controlled germination experiment with reduced water availability, suggest that SA might not affect seed performances at the establishment phase, as suggested by Xie et al. (2007). Further studies are needed to test this hypothesis under more severe stress conditions and on different species.

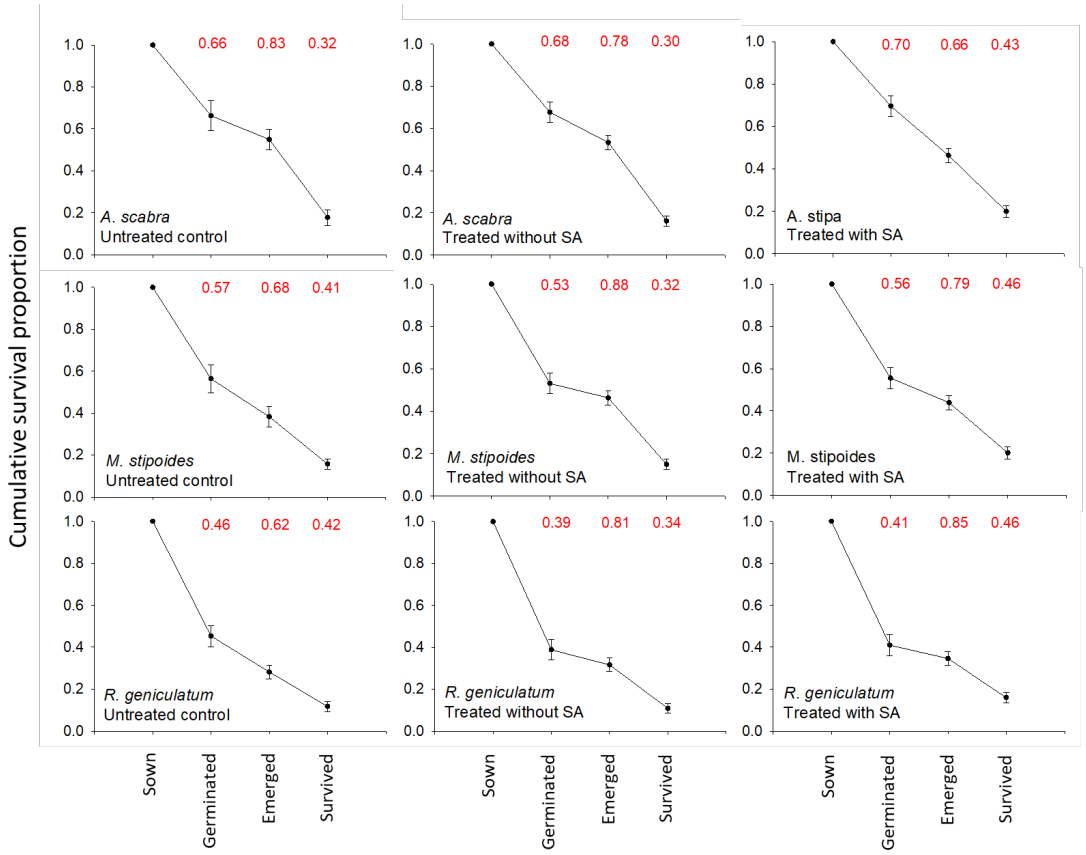



*Figure 5.6: Cumulative survival proportion of sown seeds in the line experiment (without seedling removal) through various life stages, for the three species tested without treatment, treated without SA and treated with SA. On the top of each graph, in red, are reported the probability of transitioning between life stages.*

Significant effects of SA delivering stress resistance were instead detected on the survival of established plant over the summer when seedling had to endure prolonged periods with little access to water. Total precipitation between November 2017 and February 2018, removing two major rainy events that happened over a short period (60 mm on December 20th and 147 mm on January 18th) were less than 30 mm. The effects of the summer drought were evident on the experiment where seedlings were not removed, with the probability of plant survival from an emerged seedling being

0.32 for *A. scabra*, 0.41 for *M. stipoides* and 0.42 for *R. geniculatum.* In this case, SA treated seed survived significantly better than the seed treated without SA for the three species. When considering the cumulative survival from the number of seeds initially sown, SA treatment provides a significantly higher number of plant successfully established, even in cases like *A. scabra*, when emergence of SA treated seed was lower than the seed treated without SA.

#### *5.4.3.2. SA effect on survival*

In plots where interspecific competition was removed, plant survival was higher than in the scenario when seedlings have not been removed (lines). Based on observations (no data available), the plants with removed competition were generally more developed before summer than the ones in the lines. This would have probably allowed for the development of a broader and deeper root system with better access to water during the dry summer months. Similar to the line experiment, SA treated seeds improved survival, confirming that SA exogenous application could deliver drought stress resistance (Janda et al. 2007). This improvement in survival might be due to a variety of factor, such as the effect of SA in mediating reactive oxygen species (ROS) and triggering defence-related processes (Garretón et al. 2007), and its effect on productivity and growth (Larqué-Saavedra and Martin-Mex 2007). In this study, just one of the three species tested (*M. stipoides*) showed a higher biomass production as a response to SA treatment. A previously published study reported that externally applied SA had increased root development (Gutiérrez-Coronado et al. 1998), but root growth was not evaluated in this study. Nevertheless, as this study shows, the effects of exogenous SA delivery are still present months after its application. SA absorbed through the seed (imbibing), or through emerging radicle and roots (encrusting) could be converted in SA glucoside and transferred in the vacuole where it's stored. It could be mobilized and moved through the plant after been converted in methyl salicylate, and eventually turned back to SA when needed (Park et al. 2007).

#### *5.4.3.3. Encrusting and imbibition*

When SA delivery mechanisms of imbibing and encrusting were compared in term of delivering plant survival, a significant difference was rarely detected, suggesting that seed encrusting could be used to deliver SA and its stress resistance inducing proprieties. The advantage of using SA in the seed coating processes over imbibition lies in the capability of storing seed after treatment. Seed imbibition can trigger a seed

priming effect that could improve germination speed and synchronicity in the short term (Paparella et al. 2015), but, on the other hand, accelerate seed ageing processes, reducing seed shelf-life and storability (Hussain et al. 2015). Another advantage of seed coating over imbibition is that while it delivers SA stress resistance, it can also improve seed handling and sowability, along with a wide variety of active ingredients, such as protectants, micronutrients, germination promoters and microorganism (Pedrini et al. 2017). Most of these coating treatments still need to be tested on native species for restoration, but their combined impact on seed germination, emergence, growth and plant establishment could improve the successful deployment of native seed onto degraded landscapes, ultimately allowing for a more cost-effective seed-based restoration.

## 5.5. References


Bates D, Mächler M, Bolker B, Walker S (2015) Fitting Linear Mixed-Effects Models Using lme4. J Stat Softw 67:. doi: 10.18637/jss.v067.i01

BOM (2018) Bureau of Meteorology. West Perth, WA, Australia

Bujalski W, Nienow AW (1991) Large-scale osmotic priming of onion seeds: a comparison of different strategies for oxygenation. Sci Hortic (Amsterdam) 46:13–24. doi: 10.1016/0304-4238(91)90088-G

Chojnowski M, Corbineau F, Côme D (1997) Physiological and biochemical changes induced in sunflower seeds by osmopriming and subsequent drying, storage and aging. Seed Sci Res 7:. doi: 10.1017/S096025850000372X

Conrad MK, Tischew S (2011) Grassland restoration in practice: Do we achieve the targets? A case study from Saxony-Anhalt/Germany. Ecol Eng 37:1149–1157. doi: 10.1016/j.ecoleng.2011.02.010

Costanza R, Arge R, de Groot R, et al (1997) The value of the world 's ecosystem services and natural capital. Nature 387:253–260. doi: 10.1038/387253a0

Cross AT, Stevens JC, Dixon KW (2017) One giant leap for mankind: can ecopoiesis avert mine tailings disasters? Plant Soil 421:1–5. doi: 10.1007/s11104-017-3410-y

Dat JF, Capelli N, Van Breusegem F (2007) The Interplay Between Salicylic Acid and Reactive Oxygen Species During Cell Death in Plants. In: Salicylic Acid: A Plant Hormone. pp 247–276

Durner J, Shah J, Klessig DF (1997) Salicylic acid and disease resistance in plants. Trends Plant Sci 2:266–274. doi: 10.1016/S1360-1385(97)86349-2

Gang C, Zhou W, Chen Y, et al (2014) Quantitative assessment of the contributions of climate change and human activities on global grassland degradation. Environ Earth Sci 72:4273–4282. doi: 10.1007/s12665-014-3322-6

Garretón V, Holuigue L, Salinas P, et al (2007) Salicylic Acid and Reactive Oxygen Species in the Activation of Stress Defense Genes. In: Salicylic Acid: A Plant Hormone. pp 197–246

Gibson DJ (2009) Grasses and Grassland Ecology. Oxford University Press, New York

Guan L, Scandalios JG (1995) Developmentally related responses of maize catalase genes to salicylic acid. Proc Natl Acad Sci U S A 92:5930–5934. doi: 10.1073/pnas.92.13.5930

Guan Y, Cui H, Ma W, et al (2014) An Enhanced Drought-Tolerant Method Using SA-Loaded PAMPS Polymer Materials Applied on Tobacco Pelleted Seeds. Sci World J 2014:9. doi: 10.1155/2014/752658

Guan Y, Li Z, He F, et al (2015) "On-Off" Thermoresponsive Coating Agent Containing Salicylic Acid Applied to Maize Seeds for Chilling Tolerance. PLoS One 10:e0120695. doi: 10.1371/journal.pone.0120695

Gutiérrez-Coronado MA, Trejo-López C, Larqué-Saavedra A (1998) Effects of salicylic acid on the growth of roots and shoots in soybean. Plant Physiol Biochem 36:563–565. doi: 10.1016/S0981-9428(98)80003-X

Horvat E, Gabriella S, Janda T (2007) Induction of Abiotic Stress Tolerance by Salicylic Acid Signaling. J Plant Growth Regul 26:290–300. doi: 10.1007/s00344-007-9017-4

Hussain S, Zheng M, Khan F, et al (2015) Benefits of rice seed priming are offset permanently by prolonged storage and the storage conditions. Sci Rep 5:8101. doi: 10.1038/srep08101

James JJ, Svejcar TJ, Rinella MJ (2011) Demographic processes limiting seedling recruitment in arid grassland restoration. J Appl Ecol 48:961–969. doi: 10.1111/j.1365-2664.2011.02009.x

Janda T, Horváth E, Szalai G, Páldi E (2007) Role of salicylic acid in the induction of abiotic stress tolerance. In: Salicylic Acid: A Plant Hormone. pp 91–150

Khan MIR, Fatma M, Per TS, et al (2015) Salicylic acid-induced abiotic stress tolerance and underlying mechanisms in plants. Front Plant Sci 6:. doi: 10.3389/fpls.2015.00462

Larqué-Saavedra A, Martin-Mex R (2007) Effects of Salicylic Acid on the Bioproductivity of Plants. In: Salicylic Acid: A Plant Hormone. pp 15–23

Lee S, Park C-M (2010) Modulation of reactive oxygen species by salicylic acid in Arabidopsis seed germination under high salinity. Plant Signal Behav 5:1534–1536. doi: 10.4161/psb.5.12.13159

Lewandrowski W, Erickson TE, Dixon KW, Stevens JC (2017) Increasing the germination envelope under water stress improves seedling emergence in two dominant grass species across different pulse rainfall events. J Appl Ecol 54:997–1007. doi: 10.1111/1365-2664.12816

Madsen MD, Davies KW, Williams CJ, Svejcar TJ (2012) Agglomerating seeds to enhance native seedling emergence and growth. J Appl Ecol 49:431–438. doi: 10.1111/j.1365-2664.2012.02118.x

Madsen MD, Kostka SJ, Hulet A, et al (2013) Surfactant Seed Coating – a Strategy To Improve Turfgrass Establishment on Water Repellent Soils. In: Castelani P (ed) Proceedings of the 10th International Symposium on Adjuvants for Agrochemicals, ISAA 2013, 22-26 April, Foz Do Iguaçu, Brazil, Organized Under the Auspices of ISAA Society. ISAA Society, pp 205–210

McDonald T, Jonson J, Dixon KW (2016) National standards for the practice of ecological restoration in Australia. Restor Ecol 24:S4–S32. doi: 10.1111/rec.12359

Menz MHM, Dixon KW, Hobbs RJ (2013) Hurdles and Opportunities for Landscape-Scale Restoration. Science (80- ) 339:526–527. doi: 10.1126/science.1228334

Merritt DJ, Dixon KW (2014) Seed availability for restoration. In: Bozzano M, Jalonen R, Thomas E, et al. (eds) The state of the world's forest genetic resources – thematic study: Genetic considerations in ecosystem restoration using native tree species. Food and Agriculture Organization of the United Nations, pp 97–104

Nellemann C, Corcoran E (2010) Dead Planet , Living Planet. Biodiversity and ecosystem restoration for sustainable development. United Nations Environment Programme, GRID-Arendal, Norway

O'Mara FP (2012) The role of grasslands in food security and climate change. Ann Bot 110:1263–1270. doi: 10.1093/aob/mcs209

Orrock JL, Witter MS, Reichman OJ (2008) Apparent competition with an exotic plant reduces native plant establishment. Ecology 89:1168–1174. doi: 10.1890/07-0223.1

Palmer MA, Filoso S (2009) Restoration of Ecosystem Services for Environmental Markets. Science (80- ) 325:575–576. doi: 10.1126/science.1172976

Paparella S, Araújo SS, Rossi G, et al (2015) Seed priming: state of the art and new perspectives. Plant Cell Rep 1281–1293. doi: 10.1007/s00299-015-1784-y

Park S, Kaimoyo E, Kumar D, et al (2007) Methyl salicylate is a critical mobile signal for plant systemic acquired resistance. Science 318:113–116. doi: 10.1126/science.1147113

Pearson DE, Valliant M, Carlson C, et al (2018) Spicing up restoration: can chili peppers improve restoration seeding by reducing seed predation? Restor Ecol. doi: 10.1111/rec.12862

Pedrini S, Bhalsing K, Cross AT, Dixon KW (2018a) Protocol Development Tool (PDT) for seed encrusting and pelleting. Seed Sci Technol 46:393–405. doi: 10.15258/sst.2018.46.2.21

Pedrini S, Lewandrowski W, Stevens JC, Dixon KW (2018b) Optimizing seed processing techniques to improve germination and sowability of native grasses for ecological restoration. Plant Biol. doi: 10.1111/plb.12885

Pedrini S, Merritt DJ, Stevens J, Dixon K (2017) Seed Coating: Science or Marketing Spin? Trends Plant Sci 22:106–116. doi: 10.1016/j.tplants.2016.11.002

Pyke DA (1990) Comparative demography of co-occurring introduced and native tussock grasses: persistence and potential expansion. Oecologia 82:537–543. doi: 10.1007/BF00319798

R Core Team (2015) R: A Language and Environment for Statistical Computing.itle

Raskin I, Skubatz H, Tang W, Meeuse BJD (1990) Salycilic acid levels in thermogenic and non termogenic plants. Ann Bot 66:369–373

Ritz C, Baty F, Streibig JC, Gerhard D (2015) Dose-response analysis using R. PLoS One 10:1–13. doi: 10.1371/journal.pone.0146021

Ritz C, Streibig JC, Ritz, C. & Streibig JC (2005) Bioassay Analysis using R. J Stat Softw 12:1–22. doi: 10.18637/jss.v012.i05

Rivas-San Vicente M, Plasencia J (2011) Salicylic acid beyond defence: Its role in plant growth and development. J. Exp. Bot. 62:3321–3338

Senaratna T, Merritt D, Dixon K, et al (2003) Benzoic acid may act as the functional group in salicylic acid and derivatives in the induction of multiple stress tolerance in plants. Plant Growth Regul 39:77–81. doi: 10.1023/A:1021865029762

Senaratna T, Touchell D, Bunn E, Dixon K (2000) Acetyl salicylic acid (Aspirin) and salicylic acid induce multiple stress tolerance in bean and tomato plants. Plant Growth Regul 30:157–161. doi: 10.1023/A:1006386800974

Sharafizad M, Naderi A, Ata siadat S, et al (2013) Effect of Salicylic Acid Pretreatment on Germination of Wheat under Drought Stress. J Agric Sci 5:179–199. doi: 10.5539/jas.v5n3p179

Society for Ecological Restoration International Science & Policy Working Group (2004) The SER International primer on ecological restoration. Tucson

Stevens J, Chivers I, Symons D, Dixon K (2015) Acid-digestion improves native grass seed handling and germination. Seed Sci Technol 43:313–317. doi: http://dx.doi.org/10.15258/sst.2015.43.2.19

Stevens J, Senaratna T, Sivasithamparam K (2006) Salicylic acid induces salinity tolerance in tomato (Lycopersicon esculentum cv. Roma): Associated changes in gas exchange, water relations and membrane stabilisation. Plant Growth Regul 49:77–83. doi: 10.1007/s10725-006-0019-1

Suttie JM, Reynolds SG, Batello C (2005) GRASSLANDS of the WORLD. Food and Agriculture Organization of the United Nations, Rome

Taylor AG, Allen PS, Bennett M a., et al (1998) Seed enhancements. Seed Sci Res 8:245–256. doi: 10.1017/S0960258500004141

Waters C, Whalley W, Huxtable C (2001) Grassed up. 1–50

Xie Z, Zhang ZL, Hanzlik S, et al (2007) Salicylic acid inhibits gibberellin-induced alpha-amylase expression and seed germination via a pathway involving an abscisic-acid-inducible WRKY gene. Plant Mol Biol 64:293–303. doi: 10.1007/s11103-007-9152-0

# General discussion

## Transparency is needed in the seed coating industry

The technology of seed coating is more than a century old (Mathre et al. 2001) and has become a standard procedure in the agricultural seed industry (Kaufman 1991). However, the materials, procedures, and know-how necessary to perform seed coatings are usually undisclosed and often treated as industrial secrets (Pedrini et al. 2017). Although the issue of confidentiality in the seed coating industry was acknowledged by previous publications (Taylor et al. 1998; Halmer 2008; Bennett 2016), most researchers accepted the *status quo* and performed numerous studies with outsourced and unknown seed coating formulations (e.g., Taylor et al. 1997; Grellier et al. 1999). Some degree of IP protection of intellectual propriety (IP) is justifiable to allow companies that have developed the seed coatings a return on research and development investment. However, excessive protection and lack of transparency, allowed by the dominant position of the few major players left in the market (Howard 2009), has hampered the critical evaluation of this technology, limiting the potential for innovation by emerging research teams and start-ups (Heijnen 2008). Importantly, such a constraint reduces the application to other fields such as ecological restoration where seed enhancement is relatively rare but could be of great benefit.

The literature review "Seed coating: science of marketing spin?" (Chapter 1) was the first publication arising from the thesis and the first internationally that quantified and addressed the problem by highlighting the exploitation of seed coating technologies for marketing purposes. The review provided guidance on transparency from the industry and recommended closer collaboration with academia. Furthermore, the definitions of seed coating type, materials and techniques have previously not been clearly defined and categorised, and there was no consensus across academia and industry on common definitions and nomenclature. This review provided for the first time clear definitions of otherwise redundant and confusing technical terminology, to improve clarity in this field of research. This work provided a comprehensive overview and represented a major update on seed coating technologies since the last major review was published almost twenty years ago (Taylor et al. 1998).

To address the lack of disclosure stated in the first chapter, the key tenet of this thesis is that all parts of the process, summarised in the Protocol Development Tool (PDT)

(Chapter 2), are published, as far as is practicable, in the public domain. If the advancement of seed coating technologies for improving both sustainable agriculture practices and ecological restoration is to continue, thereis a strong need to make all the information concerning the methodology and material employed in seed coating freely available. The protocol development tool (PDT), presented in Chapter 2, was developed to provide seed scientists, companies and end-users (e.g. farmers, restoration practitioners) with a standard model for the evaluation and optimisation of seed coating applicable to any species, end-use of the seed and coating type. This paper is the first published and practical, step-by-step guide for seed encrusting and pelleting.

Further customisation of the PDT is needed when applied to different equipment (e.g. rotating pan, fluidised bed), or when a combination of multiple materials are delivered to the seed. Hopefully, seed technologists will start using such a tool and develop their own seed coating capability, other than relying on the services of external companies (that require confidentiality).

## Impact of seed coating constituents on germinability of seeds

The understanding of how seed pelleting affect germination was addressed in Chapter 3. Delayed germination was detected in pelleted tomato seed, in line with what was previously described in the literature (Govinden-Soulange and Levantard 2008). However, the mechanism underlying such impediments have not been published, nor is it clear that such impacts have been previously investigated. This study highlighted how the mechanical integrity of pellets, resulting from increased binder concentration, is correlated to delay in germination, whilst water imbibition rate through the layers of pelleting material was not a factor. These findings have allowed the development of a model (trade-off analysis) to support seed technologists in assessing the best material combination to obtain pellets of the desired mechanical integrity, and to predict impacts of coating materials on germination.

The first section of the thesis has thus provided the knowledge base, developed the techniques and improved the understanding of coating materials necessary for the application of seed coating to native grass seeds, which is a major goal of the thesis.

## Application of seed enhancement to native species

The logistic impediment posed by grass seeds that limit handling, delivery to site, and render the coating process unfeasible (Guzzomi et al. 2016), was addressed in Chapter 4. The seed processing technique adopted allowed to completely remove the husk, obtaining cleaned caryopsis that germinated better than the untreated florets. This finding confirmed that the husk, due to a mechanical constraint, reduces seed germination, as previously indicated by studies on different grass species (Adkins and Simpson 1988; Bewley et al. 2013; Erickson et al. 2017). The evaluation and optimisation of the seed processing techniques of mechanical cleaning, flash flaming (Guzzomi et al. 2016) and acid digestion (Stevens et al. 2015), provided evidence in support of the acid digestion method for being the most time-effective, and for delivering superior germination outcomes. Seed processing techniques on native grass species for improving handling have usually been overlooked by practitioners, whose effort was mostly focused on designing *ad hoc* seeding apparatus that would improve in site delivery of seed with complex morphology (Loch et al. 1996). The adoption of advanced processing techniques would allow for efficient seed use through standard seeding equipment and optimise seed storage. However, before seed processing methodology can be implemented for seed storage, further research is needed to test if the processing technique are responsible for any reduction in seed viability over time. Once the acid processing method was tested and optimised, it was then applied to three test grass species, to make the coating more applicable.

Seed coating was then evaluated in Chapter 5. To allow full replicability and scalability of the study, the seed coating process was performed following the protocol development tool (Chapter 2), and to ensure the least possible detriment to germination, the coating materials were determined by the trade-off analysis described in Chapter 3. This study focused on the application of salicylic acid (SA) directly to seed via seed coating and imbibition. As expected, SA did not improve germination, but differently to what was hypothesised, it did not improve germination under water stress conditions. Seedling emergence in field condition was also unaffected by SA treatment, suggesting that SA does not provide germination enhancement (Guan and Scandalios 1995; Xie et al. 2007). However, and surprisingly given the long period of the field grow-out (10 months), SA induced stress resistance was found in plants arising from SA treated seeds, with an improvement in plant survival and growth in the field over the dry summer months. No difference was detected between SA delivered

via seed coating or imbibition, suggesting that seed coating is a viable and effective way to provide SA induced stress resistance. This study was the first to test the SA delivery of stress resistance for native seeds, and one of the first on SA delivery though seed coating (Guan et al. 2014).

## Further research

As found in this thesis, the mechanisms underpinning the physiological behaviour of coated and pelleted seed is not yet clear and requires further investigation. In Chapter 3, a correlation between mechanical resistance and delay in germination was detected. However, further testing on a wider range of species, coating materials and coating techniques is required. Moreover, a further explanation for seed coating effect on germination has been hypothesised to be oxygen availability (Taylor et al. 1997; Grellier et al. 1999). This hypothesis has not been empirically tested, due to the lack of appropriate technology for coated seed. A recently developed fluorescence-based closed-system respirometry (Tomlinson et al. 2018) could potentially be used to test this hypothesis.

This thesis focused predominantly on selected species of Australian grasses. The processing and coating techniques developed and described in this research should be applied to a wider range of grasses, with similar morphological characteristics, to understand how broadly this technology is applicable to other grass species. Furthermore, the deployment of seed coating should be expanded beyond grasses and evaluated as a tool that could overcome logistical and ecological barriers specific to particular species or restoration situations.

Of particular interest will be the use of microbiological adjuvants derived from soil biota in the coatings that could enhance plant growth, health, and survival in poor, depleted or degraded soils, by improving nutrient availability and stress resistance (including disease resistance) for the emerging seedling (Deaker et al. 2004) and as an inoculum system for improving general soil health (Maheshwari 2015). The main challenge for the application of bacteria, fungi, and actinomycetes to seed coating is to maintain the appropriate condition for bacteria survival during the seed coating process and subsequent storage (McIntyre et al. 2007). Ensuring the vitality of applied organisms is a key area that requires more detailed investigation if seed pelleting is to be used as a viable delivery system.

Another application of seed coating that could have a great impact on the use of native seed mixes is the pelleting of small-seeded species (e.g. *Melaleuca* spp., *Campanula* spp., *Calluna vulgaris*). Seed pelleting is already used on agricultural varieties with small and light seeds, like tobacco and begonia, to improve ballistic performance and precision sowing (Scott 1989). The improved handling and sowability proprieties of pelleted seed could allow for a more accurate seed-to-soil delivery system, including automated seed drills. The increase in weight and size would also reduce the risk of over-seeding and potential seed removal by wind or water.

## Implication for ecological restoration

Once effective seed coating technologies are developed and tested under laboratory conditions, issues concerning scalability of the process and cost need to be addressed. The expense to set up a seed processing and pelleting facility are high, and the extra labour required to perform and supervise these operations adds to the already high cost of native seeds (Merritt and Dixon 2011). However, seed loss and wastage during seeding operations, emergence and establishment phases should be factored in, and economic analyses should be undertaken to inform what level of improved seed success is required to make seed processing and enhancement procedures practicable and scalable (Madsen et al. 2016). Importantly, wild sourced native seed represents the major source of seed in wildland restoration. Thus, the effective use of seed pelleting and other seed enhancement technologies will overcome the ethical issues of seed wastage currently being seen in restoration seeding (Nevill et al. 2018).

Furthermore, if native seed production and use increase significantly, the economy of scale will further reduce the cost of seed enhancement technologies. In various countries, native seed markets are reaching a stage of development and sophistication where seed enhancement could be implemented. For example, Germany has a well-established and organised native seed market (Mainz and Wieden 2018) that is expected to grow significantly in the near future to address more stringent regulations. From 2020, it will be mandated that only local native seeds can be used in any revegetation or restoration involving natural areas (BNatSchG 2010). Other European countries are also in the process of developing similar markets for native seeds (De Vitis et al. 2017; Abbandonato et al. 2017; Pedrini 2018). In the United States, there is a large number of private companies producing seeds to fulfil the high demand for ecological restoration, farm-based conservation programs, roadside plantings, offset

schemes and green infrastructures (Gibson-Roy 2018). These situations present important opportunities for testing the applicability and scalability of seed coating technologies to native seeds.

# Conclusion

We are still in the early days of seed coating technologies applied to native seed, yet the potential to improve the efficiency of seed-based restoration has already been highlighted (Madsen et al. 2016; Erickson et al. 2017; Pearson et al. 2018). In this thesis, a range of techniques, materials and active ingredients were tested, in order to be applied and customised to a broader range of species where seed-to-plant conversion in direct seeding programs is low or erratic. Only in this way will it be possible to overcome logistical impediments and ecological barriers that stand in the way of successful native seed use in restoration.

The ultimate goal of this research is that advanced seed technologies will leave the laboratories and experimental field sites where they have been developed, and start being deployed at scale by native seed producers and restoration practitioners and make a contribution to the global push for recovering and restoring degraded terrestrial ecosystems worldwide (Bonn Challange 2015).

# Bibliography

Abbandonato H, Pedrini S, Pritchard HW, et al (2017) Native seed trade of herbaceous species for restoration: a European policy perspective with global implications. Restor Ecol 1–7. doi: 10.1111/rec.12641

Adkins SW, Simpson GM (1988) The physiological basis of seed dormancy in Avena fatua. IX. Characterization of two dormancy states. Physiol Plant 73:15–20. doi: 10.1111/j.1399-3054.1988.tb09186.x

Bennett GM (2016) Seed inoculation, coating and precision pelleting. Taylor and Francis group, Boca Raton FL

Bewley JD, Bradford KJ, Hilhorst HWM, Nonogaki H (2013) Seeds. Springer New York, New York, NY

BNatSchG (2010) Gesetz über Naturschutz und Land- schaftspflege (Bundesnaturschutzgesetz) amended by Article 1 of the Act of 15 June 2009, has been amended in September 2017

Bonn Challange (2015) World on track to meet ambitious forest restoration goal. http://www.bonnchallenge.org/blog/world-track-meet-ambitious-forest-restoration-goal

De Vitis M, Abbandonato H, Dixon K, et al (2017) The European Native Seed Industry: Characterization and Perspectives in Grassland Restoration. Sustainability 9:1682. doi: 10.3390/su9101682

Deaker R, Roughley RJ, Kennedy IR (2004) Legume seed inoculation technology - A review. In: Soil Biology and Biochemistry. pp 1275–1288

Erickson TE, Muñoz-rojas M, Kildisheva OA, et al (2017) Benefits of adopting seed-based technologies for rehabilitation in the mining sector: a Pilbara perspective. Aust J Bot

Gibson-Roy P (2018) Restoring grassy ecosystems - Feasible or fiction? An inquisitive Australian's experience in the USA. Ecol Manag Restor 19:11–25. doi: 10.1111/emr.12327

Govinden-Soulange J, Levantard M (2008) Comparative studies of seed priming and pelleting on percentage and meantime to germination of seeds of tomato ( Lycopersicon esculentum Mill .). African J Agric Res 3:725–731

Grellier P, Riviere LM, Renault P (1999) Transfer and water-retention properties of seed-pelleting materials. Eur J Agron 10:57–65

Guan L, Scandalios JG (1995) Developmentally related responses of maize catalase genes to salicylic acid. Proc Natl Acad Sci U S A 92:5930–5934. doi: 10.1073/pnas.92.13.5930

Guan Y, Cui H, Ma W, et al (2014) An Enhanced Drought-Tolerant Method Using SA-Loaded PAMPS Polymer Materials Applied on Tobacco Pelleted Seeds. Sci World J 2014:9. doi: 10.1155/2014/752658

Guzzomi AL, Erickson TE, Ling KY, et al (2016) Flash flaming effectively removes appendages and improves the seed coating potential of grass florets. Restor Ecol 24:S98–S105. doi: 10.1111/rec.12386

Halmer P (2008) Seed Technology and Seed Enhancement. Acta Hortic 771:17–26

Heijnen S (2008) Stephan G. Hannaford: Market Domination!: The Impact of Industry Consolidation on Competition, Innovation, and Consumer Choice. Greenwood Publishing Group

Howard PH (2009) Visualizing consolidation in the global seed industry: 1996-2008. Sustainability 1:1266–1287. doi: 10.3390/su1041266

Kaufman G (1991) Seed Coating: A tool for stand establishment; a stimulus to seed quality. Horttechnology 1:98–102

Loch DS, Johnston PW, Jensen TA, Harvey GL (1996) Harvesting, processing, and marketing Australian native grass seeds. New Zeal J Agric Res 39:591–599. doi: 10.1080/00288233.1996.9513218

Madsen MD, Davies KW, Boyd CS, et al (2016) Emerging seed enhancement technologies for overcoming barriers to restoration. Restor Ecol 24:77–84. doi: 10.1111/rec.12332

Maheshwari DK (2015) Bacterial Metabolites in Sustainable Agroecosystem

Mainz AK, Wieden M (2018) Ten years of native seed certification in Germany - A summary. Plant Biol 1–6. doi: 10.1111/plb.12866

Mathre DE, Johnston RH, Grey WE (2001) Small Grain Cereal Seed Treatment. Plant Heal Instr. doi: 10.1094/PHI-I-2001-1008-01

McIntyre HJ, Davies H, Hore TA, et al (2007) Trehalose biosynthesis in Rhizobium leguminosarum by. trifolii and its role in desiccation tolerance. Appl Environ Microbiol 73:3984–3992. doi: 10.1128/AEM.00412-07

Merritt DJ, Dixon KW (2011) Restoration Seed Banks--A Matter of Scale. Science (80- ) 332:424–425. doi: 10.1126/science.1203083

Nevill PG, Cross AT, Dixon KW (2018) Ethical seed sourcing is a key issue in meeting global restoration targets. Curr Biol 28:R1378–R1379. doi: 10.1016/j.cub.2018.11.015

Pearson DE, Valliant M, Carlson C, et al (2018) Spicing up restoration: can chili peppers improve restoration seeding by reducing seed predation? Restor Ecol. doi: 10.1111/rec.12862

Pedrini S (2018) Formation of the European native seed producers association. In: INSR news. http://ser-insr.org/news/2018/5/26/formation-of-the-european-native-seed-producers-association. Accessed 19 Dec 2018

Pedrini S, Merritt DJ, Stevens J, Dixon K (2017) Seed Coating: Science or Marketing Spin? Trends Plant Sci 22:106–116. doi: 10.1016/j.tplants.2016.11.002

Scott JM (1989) Seed coatings and treatments and their effects on plant establishment. Adv Agron 42:43–83

Stevens J, Chivers I, Symons D, Dixon K (2015) Acid-digestion improves native grass seed handling and germination. Seed Sci Technol 43:313–317. doi: http://dx.doi.org/10.15258/sst.2015.43.2.19

Taylor AG, Allen PS, Bennett M a., et al (1998) Seed enhancements. Seed Sci Res 8:245–256. doi: 10.1017/S0960258500004141

Taylor AG, Grabe DF, Paine D. (1997) Moisture content and water activity determination of pelleted and film-coated seeds. Seed Technol 19:24–32

Tomlinson S, Dalziell EL, Withers PC, et al (2018) Measuring metabolic rates of small terrestrial organisms by fluorescence-based closed-system respirometry. J Exp Biol 221:jeb172874. doi: 10.1242/jeb.172874

Xie Z, Zhang ZL, Hanzlik S, et al (2007) Salicylic acid inhibits gibberellin-induced alpha-amylase expression and seed germination via a pathway involving an abscisic-acid-inducible WRKY gene. Plant Mol Biol 64:293–303. doi: 10.1007/s11103-007-9152-0

# Appendices

## S.1. Supplemental Information: Chapter 1

To gain a better understanding of seed coating use and technological capabilities, we performed searches of the publically available material from academia and industry.

We analysed the published scientific literature since the least published major review on seed enhancement (Taylor and al. 1998) until to March 2016. The search was conducted on the primary scientific online databases searching for "seed coat*", "encrust*", "pellet*", "polymer", "germination", "emergence", "yield" and "growth". Further document searching of the bibliographies allowed for the inclusion of publication initially not detected through the database search.

For all papers selected a publication analysis was performed, that involved a full analysis of coating specifications, materials used and benefits. We then analysed the experimental outcomes, recording: species, experimental design, variables evaluated, responses assessed and benefits. Due to the diversity of experimental approaches and heterogeneity of the data, a qualitative scale was designed to represent the results based on the statistically significant difference between the coated seed compared with untreated control. In experiments with different coating treatments, to account for different outcomes, the result has been reported on a scale that incorporates all potential combinations (images S.4, S.5, S.6):

- All treatments had negative effect

-= Some treatment had negative effect, some no significant difference

= No statistically significant difference among treatments

=+ Some treatment had positive effect, some no significant difference

+ All treatments had positive effect

## *S.1.1. Seed coating/pelleting techniques*

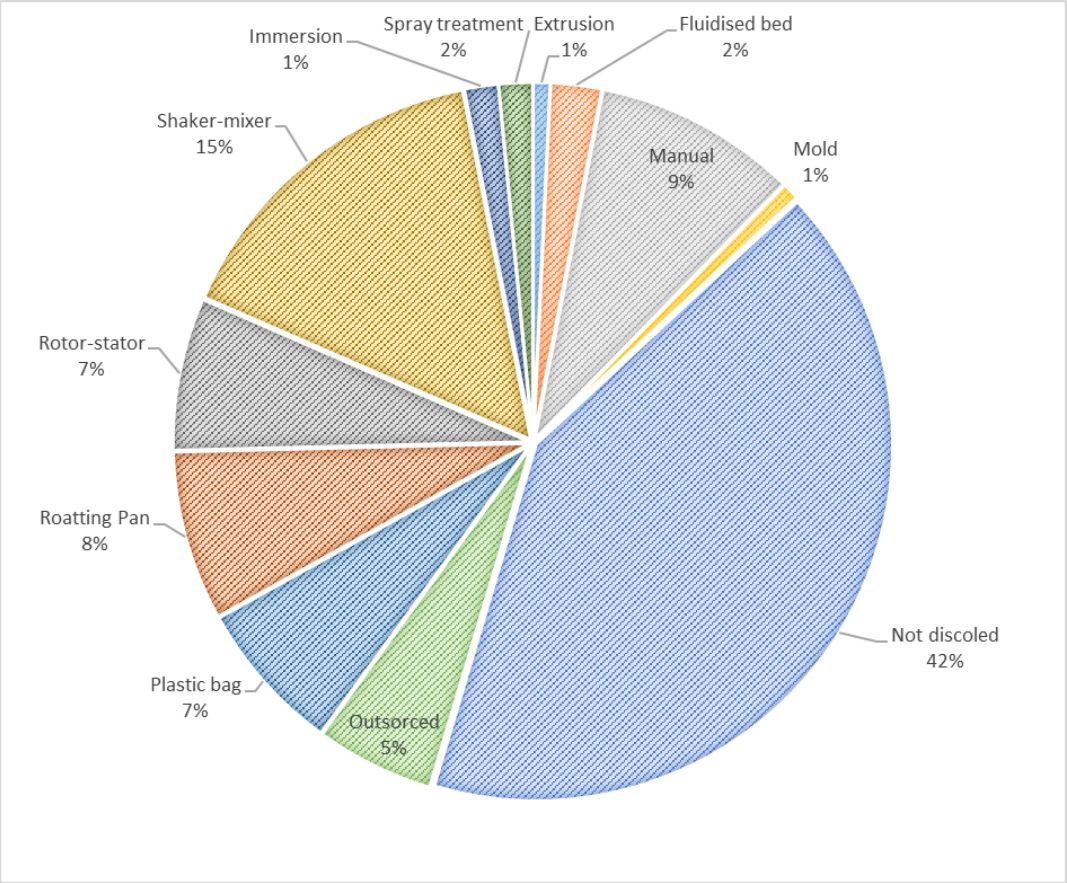



*Figure S.1: Seed coating/pelleting technique*

12 different techniques were reported in 128 publications. [2–129]

## *S.1.2. Film coating polymers and binding material*

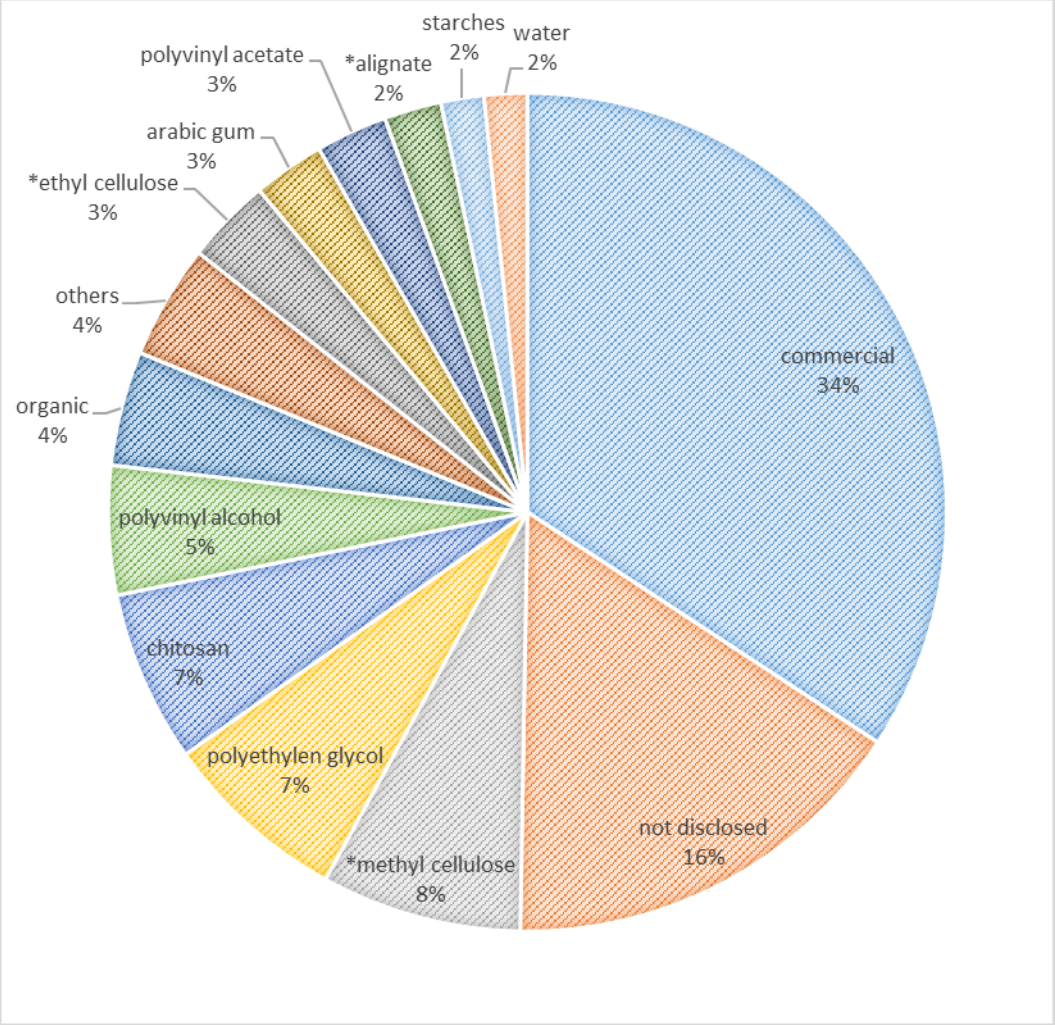



*Figure S.2: Film coating polymers and binding material.*

A total of 181 binders were reported in 127 publications. In 89 cases one binder was used, while in 38 more polymers were reported.

*methylcellulose as it is or in the form of carboxy* [21,130,84,101,89] and hydroxypropyl* [25].

*ethyl cellulose as it is or as hydroxy* [101,85,131,88]

* aglinate as it is or as sodium* [21,84,89,17], calcium* [114,54] and propylene glycol* [68]

## *S.1.3. Filler material used for encrusting and pelleting*

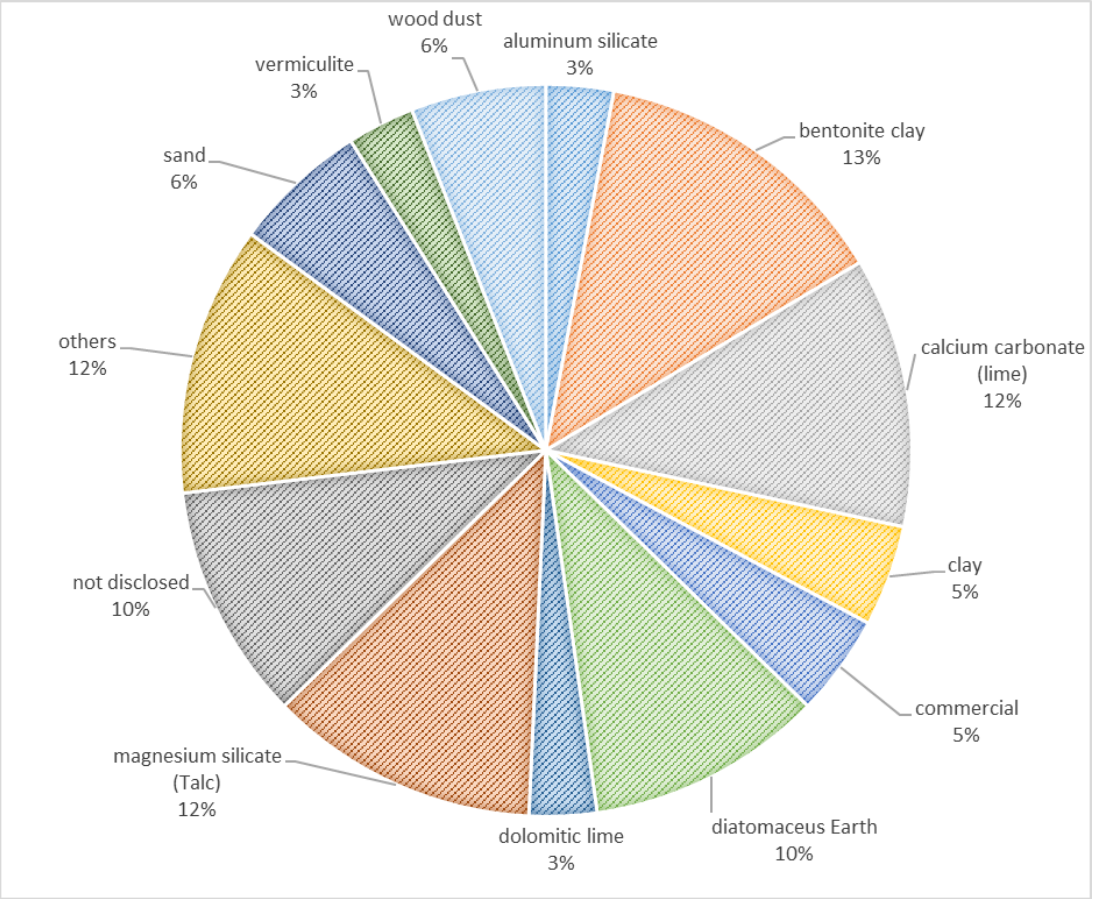



*Figure S.3: Filler material used for encrusting and pelleting.*

13 kinds of powders were reported 67 times in 44 publications. In 30 cases just one filler was used and in remaining 14 papers, more powders were reported.

## *S.1.4. Effects of seed coating with protectants*

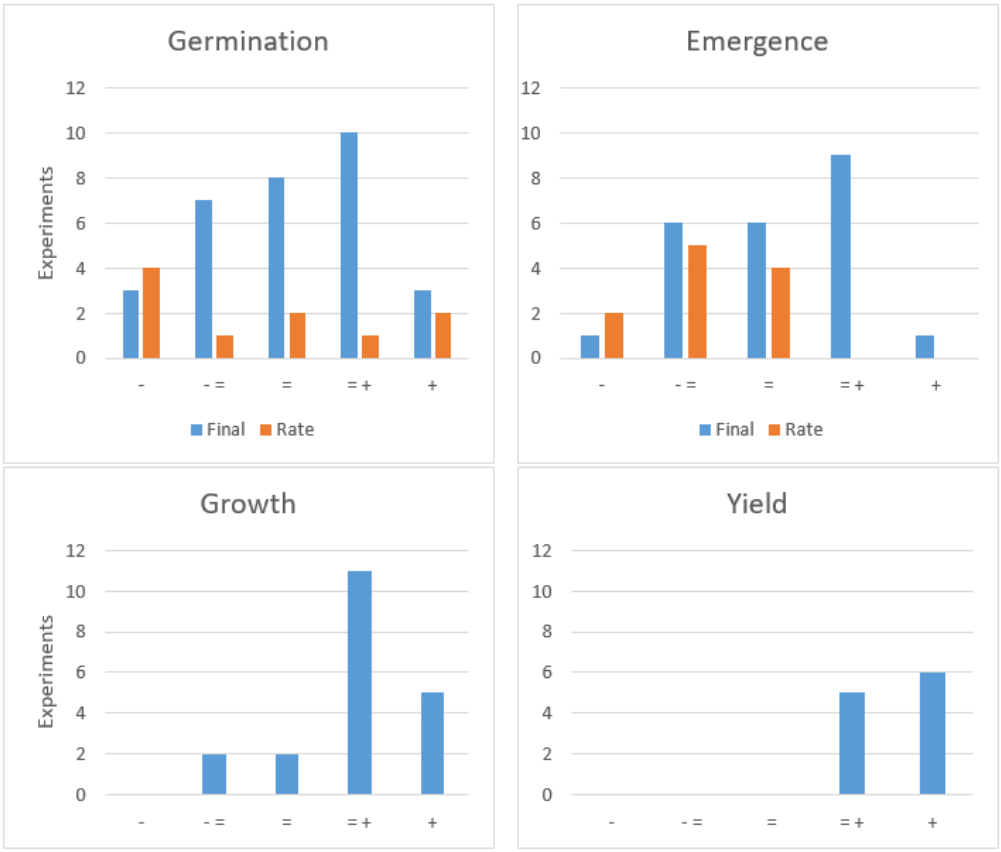



*Figure S.4: Effects of seed coating with protectants*

Protectants such as insecticide, fungicide, nematicide, pesticide, herbicide, and predator deterrent were evaluated in 43 publications for a total of 134 experiments.[7,11,19,21,25,27,28,30,43,45,49,53,57,58,61,63,64,66,67,71,72,74,76–78,80,83,85,91,93,94,101,102,104,110,110,112,113,117–119,121,129]

## *S.1.5. Effects of seed coating with nutrients.*

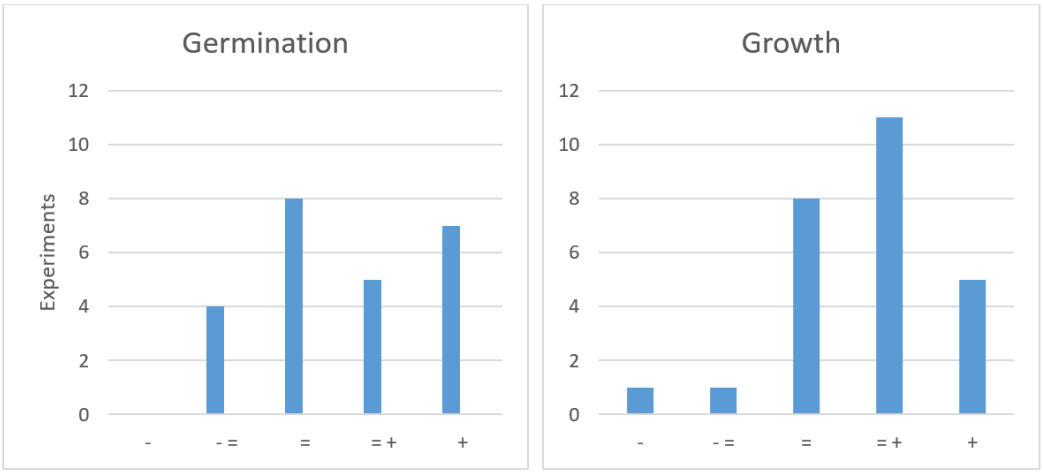



*Figure S.5: Effects of seed coating with nutrients.*

Nutrients and micronutrients were evaluated in 31 publications for a total of 85 experiments. [2,9–11,14,17,33,35,36,40,44,48,51,52,54,61–63,65,68,72,75,78,92,95,98,102,105,108,112,116]

## *S.1.6. Effects of seed coating with symbiotic organism.*

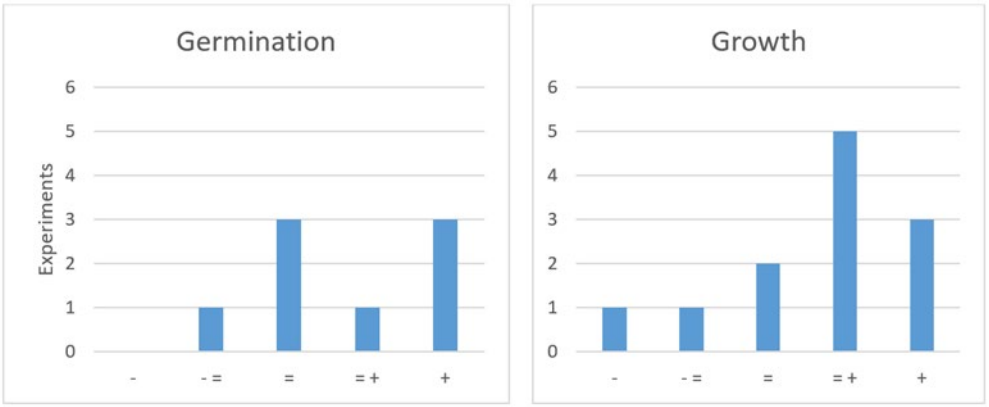



*Figure S.6: Effects of seed coating with symbiotic organism.*

Nutrients and micronutrients were evaluated in 14 publications for a total of 31 experiments. [5,7,33,55,62,69,78,95,96,105,120]

## *S.1.7. Seed coating products and services analysed divided by companies.*

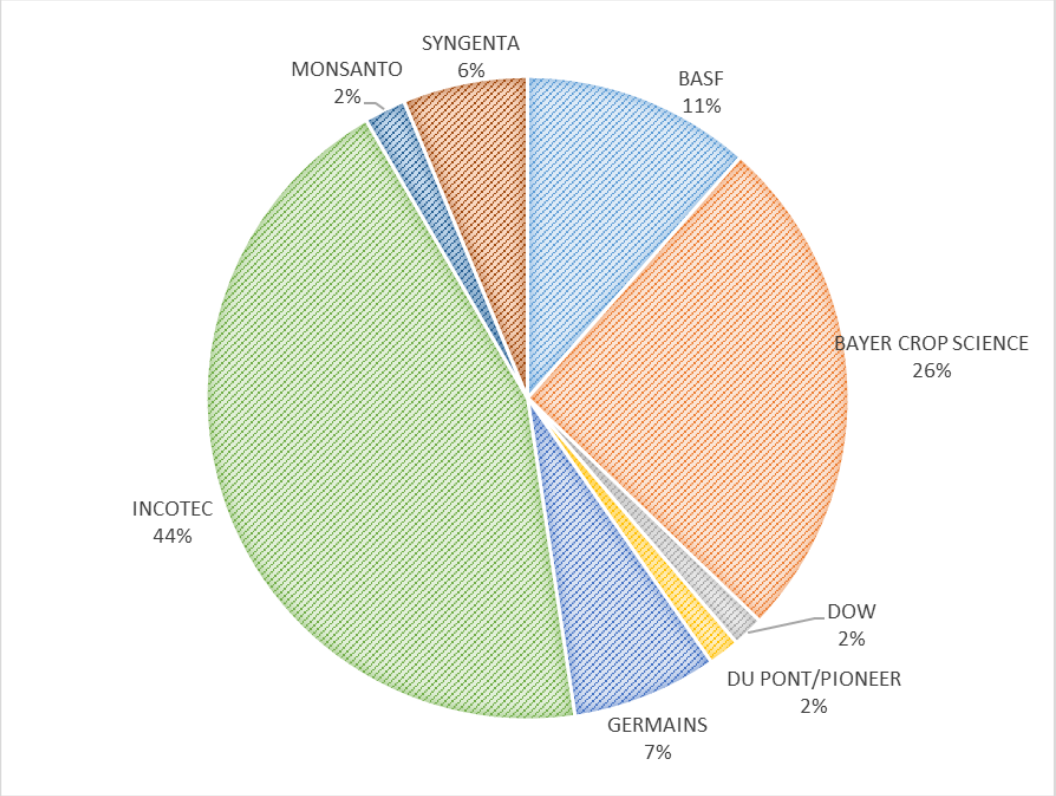



*Figure S.7: Seed coating products and services analysed divided by companies*

The commercially available seed coating products and services have been searched in the main seed technology and agrochemicals company's websites.

### *S.1.8. List of publications used in the review process*


1 Taylor, a. G. et al. Seed enhancements. , Seed Science Research, 8. (1998) , 245–256

2 Adhikari, T. et al. (2016) Zinc delivery to plants through seed coating with nano-zinc oxide particles. J. Plant Nutr. 39, 136–146

3 Williams, M.I. et al. (2016) Can biochar be used as a seed coating to improve native plant germination and growth in arid conditions? J. Arid Environ. 125, 8–15

4 Utomo, H.S. et al. (2016) Use of an Airplane and Simulated Aerial Planting to Evaluate Seed Coating, Seed Pelletization, and Seeding Rate on Smooth Cordgrass Vegetation. Ecol. Restor. 34, 10–14

5 Głodowska, M. et al. (2016) Biochar is a growth-promoting alternative to peat moss for the inoculation of corn with a pseudomonad. Agron. Sustain. Dev. 36, 21

6 Guan, Y. et al. (2015) "On-Off" Thermoresponsive Coating Agent Containing Salicylic Acid Applied to Maize Seeds for Chilling Tolerance. PLoS One 10, e0120695

7 Samac, D.A. et al. (2015) A Mineral Seed Coating for Control of Seedling Diseases of Alfalfa Suitable for Organic Production Systems. Plant Dis. 99, 614–620

8 Tripathi, B. et al. (2015) Improving Soybean Seed Performance with Natural Colorant-based Novel Seed-Coats. J. Crop Improv. 29, 301–318

9 Dmytryk, A. et al. (2015) Innovative Seed Treatment with Algae Homogenate. Waste and Biomass Valorization 6, 441–448

10 Tavares, L.C. et al. (2015) Performance of oat seeds coated with phosphorus sources and doses. Rev. Bras. Ciências Agrárias - Brazilian J. Agric. Sci. 10, 352–355

11 Lemes, E.S. et al. (2015) Response of wheat seeds to zinc application during storage. Cienc. e Investig. Agrar. 42, 21–22

12 SHINDE, P. et al. (2015) Standardization of seed coating polymer in chickpea ( Cicer arietinum L .). Karnataka J. Agric. Sci. 28, 412–413

13 Melo, A.P.C. de et al. (2015) Tomato seed coating with growing concentrations of synthetic polymer. Ciência Rural 45, 958–963

14 Deng, Q. and Zeng, D. (2015) Physicochemical Property Testing of a Novel Maize Seed Coating Agent and Its Antibacterial Mechanism Research. Open J. Soil Sci. 05, 45–52

15 Gorim, L. and Asch, F. (2015) Seed coating reduces respiration losses and affects sugar metabolism during germination and early seedling growth in cereals. Funct. Plant Biol. 42, 209

16 Sikhao, P. et al. (2015) Development of seed agglomeration technology using lettuce and tomato as model vegetable crop seeds. Sci. Hortic. (Amsterdam). 184, 85–92

17 Oliveira, S. De et al. (2014) Tratamento de sementes de Avena sativa l. com zinco: qualidade fisiológica e desempenho inicial de plantas. Semin. Ciências Agrárias 35, 1131

18 Sanchez, P.L. et al. (2014) Effects of temperature and salinity on germination of non-pelleted and pelleted guayule (Parthenium argentatum A. Gray) seeds. Ind. Crops Prod. 55, 90–96

19 Melo, A.P.C. de et al. (2014) Peliculização de sementes de tomate associada ao paclobutrazol. Bragantia 73, 123–129

20 Reis, J. et al. (2014) Germination and Vigor of Crambe Seeds Treated with Polymers under Hydric Stress. Res. J. Agron. 8, 1–5

21 Song, Y. et al. (2014) Preparation and Control Efficiency of Seed Coating Agent by Antagonistic Actinomycetes Against Clubroot. J. Agric. Sci. 6, 132–139

22 Tian, Y. et al. (2014) A novel anti-counterfeiting method: Application and decomposition of RB for broad bean seeds (Vicia faba L.). Ind. Crops Prod. 61, 278–283

23 Suma, N. et al. (2014) Influence of polymer coating on seed and seedling quality characteristics. J. Agric. Vet. Sci. 3, 486–490

24 Carvajal, M. et al. (2014) Seed Coating Increase Broccoli Nutrient Content and Availability after Cooking. J. Agric. Sci. 7, 182–191

25 Keawkham, T. et al. (2014) Effect of polymer seed coating and seed dressing with pesticides on seed quality and storability of hybrid cucumber. Aust. J. Crop Sci. 8, 1–14

26 Guan, Y. et al. (2014) An Enhanced Drought-Tolerant Method Using SA-Loaded PAMPS Polymer Materials Applied on Tobacco Pelleted Seeds. Sci. World J. 2014, 9

27 Yang, D. et al. (2014) Microencapsulation of seed-coating tebuconazole and its effects on physiology and biochemistry of maize seedlings. Colloids Surfaces B Biointerfaces 114, 241–246

28 Castañeda, L.M.F. et al. (2014) Innovative Rice Seed Coating ( Oryza Sativa ) with Polymer Nanofibres and Microparticles Using the Electrospinning Method. J. Res. Updat. Polym. Sci. 55, 33–39

29 Verma, O. and Verma, R. (2014) Effect of seed coating material and storage containers on Germination and seedling Vigour of soybean (Glycine max L.). SAARC J. Agric. 12, 16–24

30 Corlett, F.M.. et al. (2014) The influence of seed coating on the vigor and early seedling growth of barley. Cienc. e Investig. Agrar. 41, 25–26

31 Madsen, M.D. et al. (2014) Improving Restoration of Exotic Annual Grass-Invaded Rangelands Through Activated Carbon Seed Enhancement Technologies. Rangel. Ecol. Manag. 67, 61–67

32 Tian, Y. et al. (2013) Application of fluorescent dyes for falsification-preventing of pea seeds (Pisum sativum L.). Aust. J. Crop Sci. 7, 147–151

33 Tavares, L.C. et al. (2013) Potássio via recobrimento de sementes de soja: efeitos na qualidade fi siológica e no rendimento. Ciência Rural 43, 1196–1202

34 Lizárraga-Paulín, E.-G. et al. (2013) Maize seed coatings and seedling sprayings with chitosan and hydrogen peroxide: their influence on some phenological and biochemical behaviors. J. Zhejiang Univ. Sci. B 14, 87–96

35 Wiatrak, P. (2013) Influence of seed coating with micronutrients on growth and yield of winter wheat in southeastern coastal plains. Am. J. Agric. Biol. Sci. 8, 230–238

36 Rehman, A.U. and Farooq, M. (2013) Boron application through seed coating improves the water relations, panicle fertility, kernel yield, and biofortification of fine grain aromatic rice. Acta Physiol. Plant. 35, 411–418

37 Guan, Y. et al. (2013) The Novel Approach to Enhance Seed Security: Dual Anti-Counterfeiting Methods Applied on Tobacco Pelleted Seeds. PLoS One 8, e57274

38 Rushing, J.B. et al. (2013) Seed Safening from Herbicidal Injury in Switchgrass Establishment. Crop Sci. 53, 1650

39 Guan, Y. et al. (2013) Pathway to keep seed security: The application of fluorescein to identify true and fake pelleted seed in tobacco. Ind. Crops Prod. 45, 367–372

40 Wiatrak, P. (2013) Effect of polymer seed coating with micronutrients on soybeans in southeastern coastal plains. Am. J. Agric. Biol. Sci. 8, 302–308

41 Gevrek, M.N. et al. (2012) Growth and Yield Response of Rice ( Oryza sativa ) to Different Seed Coating Agents. Int. J. Agric. Biol. 14, 826–830

42 Serena, M. et al. (2012) Turfgrass Establishment from Polymer-coated Seed Under Saline Irrigation. HortScience 47, 1789–1794

43 Zeng, D. et al. (2012) Preparation and Study of a Novel, Environmentally Friendly Seed-Coating Agent for Wheat. Commun. Soil Sci. Plant Anal. 43, 1490–1497

44 Wang, E.W. (2012) Research on the Effect of Rape Seed Germination Based on Environment Friendly Seed Coating Mixing Different Potassium Fertilizers. Appl. Mech. Mater. 246–247, 648–652

45 Tavares, L.C. et al. (2012) Performance of lowland rice seeds coated with dolomitic limestone and aluminum silicate. Rev. Bras. Sementes 34, 202–211

46 Klafke, A.V. et al. (2012) Desempenho de sementes nuas e revestidas de azevém-anual em condições de estresse salino. Rev. Bras. Zootec. 41, 1093–1099

47 Serena, M. et al. (2012) Media selection and seed coating influence germination of turfgrasses under salinity. HortScience 47, 116–120

48 Khunkeaw, S. et al. (2012) Using Urea Formaldehyde and Polyethyleneglycol as Seed Coating to Improve Maize Seed Qualities. Chiang Mai Univ. J. Nat. Sci. 11, 257–262

49 Gesch, R.W. et al. (2012) Can using polymer-coated seed reduce the risk of poor soybean emergence in no-tillage soil? F. Crop. Res. 125, 109–116

50 Madsen, M.D. et al. (2012) Agglomerating seeds to enhance native seedling emergence and growth. J. Appl. Ecol. 49, 431–438

51 Rehman, A.U. et al. (2012) Optimizing the Boron Seed Coating Treatments for Improving the Germination and Early Seedling Growth of Fine Grain Rice. Int. J. Agric. Biol. 14, 453–456

52 Sochorec, M. and Knot, P. (2012) The effect of fertilizer seed coating on the germinating capacity and initial development of some turf grass species and white clover. Acta Univ. Agric. … 60, 199–204

53 Avelar, S.A.G. et al. The use of film coating on the performance of treated corn seed. , Revista Brasileira de Sementes, 34. (2012) , 186–192

54 Pilar-Izquierdo, M.C. et al. (2012) Barley seed coating with free and immobilized alkaline phosphatase to improve P uptake and plant growth. J. Agric. Sci. 150, 691–701

55 Domaradzki, M. et al. (2012) The Application of agglomerative granulation for seeds. Part 2. Pelleting of organic seeds. CHEMIK 66, 473–478

56 Gorim, L. and Asch, F. (2012) Effects of Composition and Share of Seed Coatings on the Mobilization Efficiency of Cereal Seeds During Germination. J. Agron. Crop Sci. 198, 81–91

57 Zeng, D. et al. (2012) Application of Bioactive Coatings Based on Chitosan for Soybean Seed Protection. Int. J. Carbohydr. Chem. 2012, 1–5

58 Zeng, D.F. and Mei, X. (2011) Application of natural aminopolysaccharide in seed film-coating for pest control and cotton growth. Russ. Agric. Sci. 37, 20–24

59 Arshadi, J. and Asgharipour, M.R. (2011) The Effects of Seed Size on Germination and Early Seedling Growth of Pelleted Seeds. J. Appl. Sci. Res. 7, 1257–1260

60 Salanenka, Y. a. and Taylor, A.G. (2011) Seedcoat permeability: Uptake and post-germination transport of applied model tracer compounds. HortScience 46, 622–626

61 Zeng, D. and Tu, R. (2011) Preparation and study of an environmentally friendly seed-coating agent for cucumber. Front. Agric. China 5, 328–332

62 Geetha, V.V. and Balamuruga, P. (2011) Organic Seed Pelleting in Mustard. Res. J. Seed Sci. 4, 174–180

63 Avelar, S.A.G. et al. (2011) Armazenamento de sementes de soja tratadas com fungicida , inseticida e micronutriente e recobertas com polímeros líquido e em pó. Ciência Rural 41, 1719–1725

64 Ludwig, M.P. et al. (2011) Eficiência do recobrimento de sementes de soja em equipamento com sistema de aspersão. Ciência Rural 41, 557–563

65 Zeng, D. and Wang, H. (2010) Preparation of a Novel Highly Effective and Environmental Friendly Wheat Seed Coating Agent. Agric. Sci. China 9, 937–941

66 Zeng, D.-F. and Zhang, L. (2010) A novel environmentally friendly soybean seed-coating agent. Acta Agric. Scand. Sect. B - Soil Plant Sci. 60, 545–551

67 Ziani, K. et al. (2010) Application of bioactive coatings based on chitosan for artichoke seed protection. Crop Prot. 29, 853–859

68 John, R.P. et al. (2010) Development of emulsion from rhizobial fermented starch industry wastewater for application as Medicago sativa seed coat. Eng. Life Sci. 10, 248–256

69 Liu, Y. et al. (2010) Effect of seed coating on plant growth and soil conditions: A preliminary study for restoration of degraded rangeland in the Qinghai-Tibetan Plateau, China. Grassl. Sci. 56, 145–152

70 Leinauer, B. et al. (2010) Seed Coating and Seeding Rate Effects on Turfgrass Germination and Establishment. HortScience 20, 179–185

71 Richardson, M.D. and Hignight, K.W. (2010) Seedling emergence of tall fescue and kentucky bluegrass, as affected by two seed coating techniques. Horttechnology 20, 415–417

72 Zeng, D. et al. Study and Preparation of an Environmentally Friendly Corn Seed Coating Agent. , Journal of Plant Protection Research, 50. (2010) , 210–214

73 Westcott, M. et al. (2010) The evaluation of the effect of seed coating on the germination of three perennial grass seed types in different growth mediums. South African J. Bot. 76, 406–407

74 SANTOS, F.C. et al. (2010) Tratamento Químico , Revestimento e Armazenamento de Sementes de Brachiaria brinzantha cv. Marandu. Rev. Bras. Sementes 32, 69–79

75 KARANAM, P. V. and VADEZ, V. (2010) PHOSPHORUS COATING ON PEARL MILLET SEED IN LOW P ALFISOL IMPROVES PLANT ESTABLISHMENT AND INCREASES STOVER MORE THAN SEED YIELD. Exp. Agric. 46, 457–469

76 Thobunluep, P. (2009) The Inhibitory Effect of the Various Seed Coating Substances Against Rice Seed Borne Fungi and their Shelf-Life during Storage. Pakistan J. Biol. Sci. 12, 1102–1110

77 Thobunluepop, P. et al. (2009) The perspective effects of various seed coating substances on rice seed variety khao dawk mali 105 storability II: The case study of chemical and biochemical properties. Pakistan J. Biol. Sci. 12, 574–581

78 Diniz, K.A. et al. (2009) Sweet pepper seed responses to inoculation with microorganisms and coating with micronutrients, aminoacids and plant growth regulators, 66

79 Oliveira, A.F. de and Soldi, V. (2009) Preparação, caracterização e propriedades de filmes poliméricos com potencial aplicação no recobrimento de sementes. Quim. Nova 32, 1845–1849

80 Zeng, D. and Shi, Y. (2009) Preparation and application of a novel environmentally friendly organic seed coating for rice. J. Sci. Food Agric. 89, 2181–2185

81 Kavak, S. and Eser, B. (2009) Influence of polymer coatings on water uptake and germination of onion (Allium cepa L. cv. Aki) seeds before and after storage. Sci. Hortic. (Amsterdam). 121, 7–11

82 Nascimento, W.M. et al. (2009) Germinação de sementes de cenoura osmoticamente condicionadas e peletizadas com diversos ingredientes. Hortic. Bras. 27, 12–16

83 Pereira, C.E. et al. (2009) Tratamento fungicida via peliculização e inoculação de bradyrhizobium em sementes de soja. Rev. Ciência Agronômica 40, 433–440

84 Pilar, M.C. et al. (2009) Alkaline phosphatase-polyresorcinol complex: characterization and application to seed coating. J. Agric. Food Chem. 57, 1967–1974

85 Nisar, K. et al. (2009) Pesticidal seed coats based on azadirachtin-A: release kinetics, storage life and performance. Pest Manag. Sci. 65, 175–82

86 Murata, M.R. et al. (2008) Pelleting or Priming Seed with Calcium Improves Groundnut Seedling Survival in Acid Soils. J. Plant Nutr. 31, 1736–1745

87 Salanenka, Y.A. and Taylor, A.G. (2008) Seed Coat Permeability and Uptake of Applied Systemic Compounds. Acta Hortic. 782, 151–154

88 Almeida, C. de and Rocha, S.C.S. (2008) Eficiência do processo de recobrimento de sementes de brócolos recobertas com polímero em leito de jorro. Eng. Agrícola 28, 305–314

89 Honglu, X. and Guomei, X. (2008) Suspension Property of Gemini Surfactant in Seed Coating Agent. J. Dispers. Sci. Technol. 29, 496–501

90 Z. B. Barut (2008) Seed Coating and Tillage Effects on Sesame Stand Establishment and Planter Performance for Single Seed Sowing. Appl. Eng. Agric. 24, 565–571

91 Govinden-Soulange, J. and Levantard, M. (2008) Comparative studies of seed priming and pelleting on percentage and meantime to germination of seeds of tomato ( Lycopersicon esculentum Mill .). African J. Agric. Res. 3, 725–731

92 Mašauskas, V. et al. (2008) Phosphorus seed coating as starter fertilization for spring malting barley. Acta Agric. Scand. Sect. B - Plant Soil Sci. 58, 124–131

93 Sharratt, B.S. and Gesch, R.W. (2008) Emergence of Polymer-Coated Corn and Soybean Influenced by Tillage and Sowing Date. Agron. J. 100, 585

94 Dawar, S. et al. (2008) Effect of seed coating material in the efficacy of microbial antagonists for the control of root rot fungi on Okra and Sunflower. Pakistan J. Bot. 40, 1269–1278

95 Kubota, A. et al. (2008) Effect of fertilizer-N application and seed coating with rhizobial inoculants on soybean yield in eastern Paraguay. Rev. Bras. Ciência do Solo DOI: 10.1590/S0100-06832008000400027

96 Conceição, P.M. da et al. (2008) Recobrimento de sementes de milho com ácidos húmicos e bactérias diazotróficas endofíticas. Pesqui. Agropecuária Bras. 43, 545–548

97 Mendonça, E.A.F. de et al. (2007) Revestimento de sementes de milho superdoce (sh2). Rev. Bras. Sementes 29, 68–79

98 Diniz, K.A. et al. (2007) Qualidade fisiológica e atividade enzimática de sementes de alface revestidas com diferentes doses de micronutrientes, aminoácidos e reguladores de crescimento. Rev. Ciência Agronômica 38, 396–400

99 Mangold, J.M. and Sheley, R.L. (2007) Effects of Soil Texture , Watering Frequency , and a Hydrogel on the Emergence and Survival of Coated and Uncoated Crested Wheatgrass Seeds. Ecol. Restor. 25, 6–11

100 ZHANG, H. et al. (2007) Effect and Mechanism of Cold Tolerant Seed-Coating Agents on the Cold Tolerance of Early Indica Rice Seedlings. Agric. Sci. China 6, 792–801

101 Kumar, J. et al. (2007) Development of polymeric seed coats for seed quality enhancement of soybean. Indian J. Agric. Sci. 77, 738–743

102 Bays, R. et al. Recobrimento de sementes de soja com micronutrientes, fungicida e polímero. , Revista Brasileira de Sementes, 29. (2007) , 60–67

103 Song, L. and Kalms, I. (2007) Improving germination of tropical grasses with new seed-coating technology. Trop. Grasslands 41, 242

104 Medeiros, E.M. et al. (2006) Recobrimento de sementes de cenoura com aglomerante em diversas proporções e fungicida. Rev. Bras. Sementes 28, 94–100

105 Diniz, K.A. et al. (2006) Incorporação de microrganismos, aminoácidos, micronutrientes e reguladores de crescimento em sementes de alface pela técnica de peliculização. Rev. Bras. Sementes 28, 37–43

106 Turner, S.R. et al. (2006) Influence of polymer seed coatings, soil raking, and time of sowing on seedling performance in post-mining restoration. Restor. Ecol. 14, 267–277

107 Esfahani, M.N. and Shariati, M. (2006) The use of pelletin in order to delay germination on Trifolium repens. Pakistan J. Biol. Sci. 9, 893–897

108 Peltonen-Sainio, P. et al. (2006) Phosphorus seed coating enhancement on early growth and yield components in oat. Agron. J. 98, 206–211

109 ARSEGO, O. et al. (2006) Recobrimento de sementes de arroz irrigado com ácido giberélico, fungicidas e polímero. Rev. Bras. Sementes 28, 96–100

110 Mazibuko, T.G. and Modi, a. T. (2005) Comparison of osmopriming and seed coating with calcium salts for green bean performance under field conditions. II. Stand establishment, chlorophyll fluorescence and yield. South African J. Plant Soil 22, 16–21

111 Qiu, J. et al. (2005) Seed film coating with uniconazole improves rape seedling growth in relation to physiological changes under waterlogging stress. Plant Growth Regul. 47, 75–81

112 John, S.S. et al. (2005) Seed Film Coating Technology for Maximizing the Growth and Productivity of Maize. Karnataka J. Agric. Sci. 18,

113 Gonçalves Barros, R. et al. (2005) Efeito do armazenamento na compatibilidade de fungicidas e inseticidas, associados ou não a um polímero no tratamento de sementes de feijão. Bragantia 64, 459–465

114 Sarrocco, S. et al. (2004) Seeds encapsulation in calcium alginate pellets. Seed Sci. Technol. 32, 649–661

115 Taylor, A.G. et al. (2004) Coating technologies for seed treatment applications. Acta Hortic. 631, 49–54

116 Duarte, C.R. et al. (2004) Experimental study and simulation of mass distribution of the covering layer of soybean seeds coated in a spouted bed. Brazilian J. Chem. Eng. 21, 59 – 67

117 Johnson, E.N. et al. (2004) Seeding date and polymer seed coating effects on plant establishment and yield of fall-seeded canola in the Northern Great Plains. Can. J. Plant Sci. 84, 955–963

118 Clayton, G.W. et al. (2004) Polymer seed coating of early- and late-fall-seeded herbicide-tolerant canola ( Brassica napus L .) cultivars. Can. J. Plant Sci. 84, 971–979

119 Willenborg, C.J. et al. (2004) Germination Characteristics of Polymer-Coated Canola ( L.) Seeds Subjected to Moisture Stress at Different Temperatures. Agron. J. 96, 786

120 Hartley, E. et al. (2004) Lime pelleting inoculated serradella (Ornithopus spp.) increases nodulation and yield. Soil Biol. Biochem. 36, 1289–1294

121 Kanampiu, F.K. et al. Multi-site, multi-season field tests demonstrate that herbicide seed-coating herbicide-resistance maize controls Striga spp. and

increases yields in several African countries. , Crop Protection, 22. (2003) , 697–706

122 Ram, M.S. et al. (2002) Invisible coatings for wheat kernels. Cereal Chem. 79, 857–860

123 Hirano, S. et al. (2001) Soybean seeds surface-coated with depolymerised chitins: Chitinase activity as a predictive index for the harvest of beans in field culture. J. Sci. Food Agric. 81, 205–209

124 Tweddell, R.J. et al. (2000) A two-year field study of a commercial biostimulant applied on maize as seed coating. Can. J. Plant Sci. 80, 805–807

125 Charnay, M.P. et al. (2000) Influence of soil type and water content on release of triticonazole from coated maize seed. Pest Manag. Sci. 56, 249–256

126 Greipsson, S. (1999) Seed coating improves establishment of surface seeded Poa pratensis used in revegetation. Seed Sci. Technol. 27, 1029–1032

127 Korpal, W. (1999) Investigations of the influence of component characteristics of a coat on the germination ability of coated seeds. Int. Agrophysics 13, 463–468

128 Steffen, R. et al. (1999) Effect of two seed treatment coatings on corn planter seeding rate and monitor accuracy. Appl. Eng. Agric. 15, 605–608

129 Johnson, G.A. et al. (1999) , Use of temperature-responsive polymer seed coating to control seed germination. , in Acta Horticulturae, 504, pp. 229–236

130 Yang, H. et al. (2011) , Study and application of seed-coating. , in International Conference on New Technology of Agricultural Engineering, pp. 150–152

131 Almeida, C. De et al. Polymer coating, germination and vigor of broccoli seeds. , Scientia Agricola, 62. (2005) , 221–226

# S.2. Activities related to this thesis

## *S.2.1. Publications*

**The European Native Seed Industry: Characterization and Perspectives in Grassland Restoration**

*De Vitis Marcello, Abbandonato Holly, Dixon Kingsley, Laverack Giles, Bonomi Costantino and Pedrini Simone*

ABSTRACT

The European Union committed to restore 15% of degraded ecosystems by 2020, and to comply with this goal, native plant material, such as seeds, is needed in large quantities. The native seed production of herbaceous species plays a critical role in supplying seed for restoration of a key ecosystem: grasslands. The objective of this work is to provide for the first time a characterization of the sector at a multi-country European level together with key information about the community of native seed users via intensive web-based research and a direct survey of industry participants. Based on more than 1300 contacts and direct surveying of more than 200 stakeholders across Europe, responses indicated that: the European native seed industry consists primarily of small to medium enterprises; responding native seed users purchase annually an average of 3600 kg of seeds with an average expenditure of €17,600; the industry (suppliers and consumers) favours development of seed zones and would participate in a European network for knowledge sharing. This study provides framework principles that can guide decisions in this sector, critical for fulfilling the growing demand for native seed as a primary tool for large-scale restoration on the continent.

AUTHOR CONTRIBUTIONS

Marcello De Vitis, Holly Abbandonato, Giles Laverack, Costantino Bonomi and Simone Pedrini conceived the study; Marcello De Vitis and Holly Abbandonato gathered international contacts, and designed and circulated the survey; Marcello De Vitis collected and analysed the data and wrote the paper; Holly Abbandonato, Kingsley W. Dixon, Giles Laverack, Costantino Bonomi and Simone Pedrini provided substantial

editing and revision to the manuscript draft. All authors have read and approved the final manuscript

REFERENCE

De Vitis M, Abbandonato H, Dixon K, et al (2017) The European Native Seed Industry: Characterization and Perspectives in Grassland Restoration. Sustainability 9:1682. doi: 10.3390/su9101682

**Native seed trade of herbaceous species for restoration: a European policy perspective with global implications**

*Abbandonato Holly, Pedrini Simone, Pritchard Hugh, De Vitis Marcello and Bonomi Costantino*

ABSTRACT

With the need to meet ambitious restoration targets, an improved native seed sector for the production of herbaceous species with a practical and supportive policy framework is recognized. We evaluated the current "ready-made" policy frameworks in Europe regarding the native seed supply of herbaceous species and found them to be, generally, unsatisfactory for both producers and users. Initially, such policies were designed for fodder seed and relate to distinctness, uniformity, and stability, traits that do not reflect the genetic heterogeneity of native species required for ecological restoration. Until recently, more suitable certification standards were designed to multiply fodder seed for preservation of the natural environment; however, due to the disparateness of the seed market in Europe, this policy is rarely practical and fails to encompass all herbaceous native species often resulting in unregulated seed sales. We recommend a new or adapted native seed policy constructed through a participatory or bottom-up approach and supported through the formation of widely based trade associations. Such a policy could stimulate the native seed trade with concomitant impacts on the speed of improving ecosystem services. Key words: bottom-up approach, certification, fodder seed, native seed production, seed policy, seed quality Implications for Practice

• When multiple stakeholders are involved, a participatory or bottom-up approach should be used to adapt or devise a new native seed policy for restoration.

• Native seed policy should start by being applicable to all species to prevent the sale of seeds of unknown origin and quality.

• Member states can modify regulations based on the development of their seed market.

• Native seed regulations need to focus on protecting genetic integrity by applying certification procedures that are not agriculturally based (distinctness, uniformity, and stability).

• Quantitative restrictions in seed policies limit market expansion and do not facilitate the demand for large quantities of herbaceous native seed for ecological restoration.

## AUTHOR CONTRIBUTIONS

Holly Abbandonato, and Costantino Bonomi conceived the purpose of the study reported; Holly Abbandonato was the lead writer and designer; Holly Abbandonato, and Marcello De Vitis designed the figures and tables; and manuscript revision was given by Simone Pedrini, Hugh Pritchard, Marcello De Vitis, and, Costantino Bonomi.

## REFERENCE

Abbandonato H, Pedrini S, Pritchard HW, et al (2017) Native seed trade of herbaceous species for restoration: a European policy perspective with global implications. Restor Ecol 1–7. doi: 10.1111/rec.12641

### *S.2.2. Conference presentations*

Pedrini S (2018) The (re)discovery of seed coating: the brief journey of seed enhancement from crop farms to restoration site. Centre for Mine Site Restoration annual workshop 2018, Perth (AUS) 19-20/11/2018

Pedrini S (2018) The Past, present and future of native seed market in Europe. Society for Ecological Restoration Australasia SERA2018, Brisbane (AUS) 25-28/09/2018

Pedrini S (2018) Optimisation of seed coating technology to native grasses. Society for Ecological Restoration Australasia SERA2018, Brisbane (AUS) 25-28/09/2018

Pedrini S, Lewandrosky W, Merritt D, Stevens J, Dixon K (2017) Optimizing seed processing techniques to improve germination and sowability of native grasses for restoration: implications for temperate grassland ecosystems. Seed Quality of Native Species - ecology, production & policy, Kew (GB), 27/09/2017

Pedrini S, Merritt D, Stevens J, Cross A, Dixon K (2017) Seed enhancement for ecological restoration - SER 2017 World Conference on Ecological Restoration, Foz do Iguassu, (BR): 30/08/2017

Pedrini S, Merritt D, Dixon K (2017) Innovation in seed technology, Pilbara Rehabilitation Group, Perth (AUS): 16/07/2017

Pedrini S, Merritt D, Dixon K (2016) Restoration: Increasing success with seeding programs. Seed coating and flaming. Native Grass Researchers Workshop 2016. Melbourne (AUS): 17/11/2016

Pedrini S, Merritt D, Dixon K (2016) Native seed down under: What's going on in Australia. Nasstec Outreach Workshop. Trento (IT): 27/09/2016

Pedrini S, Merritt D, Dixon K (2016) Seed enablement technologies: smart seed for restoration. National Seed Science Forum, Mount Annan Botanic Garden (AUS) Australia: March 2016

Pedrini S, Merritt D, Dixon K (2015) Seed enablement technologies for automated forest restoration. Automated Forest Restoration. Chiang Mai, (THA), 28/10/2015

### *S.2.3. Interactive Recovery Wheel App*

The concept of the recovery wheel as a visual tool to help restoration practitioners assessing the recovery level of a site/habitat undergoing ecological restoration, and provide a 5-star rating system, was firstly introduced in the National Standards for the practice of ecological restoration (McDonald et al. 2016). It was later accepted, with slight attributes modifications, by the International Standards (Mcdonald et al. 2016)

The clarity and simplicity of interpretation of the recovery wheel would make it the preferred tool for reporting and presenting to the public, stakeholders, and funding bodies the outcomes of any given restoration project.

The recovery wheel can be used at different stages of a restoration project. In the planning phase, the recovery wheel would help to identify the attributes that need prioritisation, and forecast the desired outcomes of the restoration project. During the management and monitoring phase, it can be used to assess the trajectory, and recognise the attributes that need to be improved, to achieve, or maintain, the best feasible restoration outcome.

However, the recovery wheel originally presented in the "standards", was available just in printable form (pdf) as a Blank progress assessment template (annexe 5) providing the table "evaluation of ecosystem recovery pro-forma" and the "recovery wheel". The template needed to be printed and manually filled by the operator, reducing its practicality on site, and potentially limiting its use by practitioners.

THE VIDEO FOR THIS APPLICATION IS VISIBLE AT THE FOLLOWING LINK:
https://youtu.be/X2z5UoQLzmQ

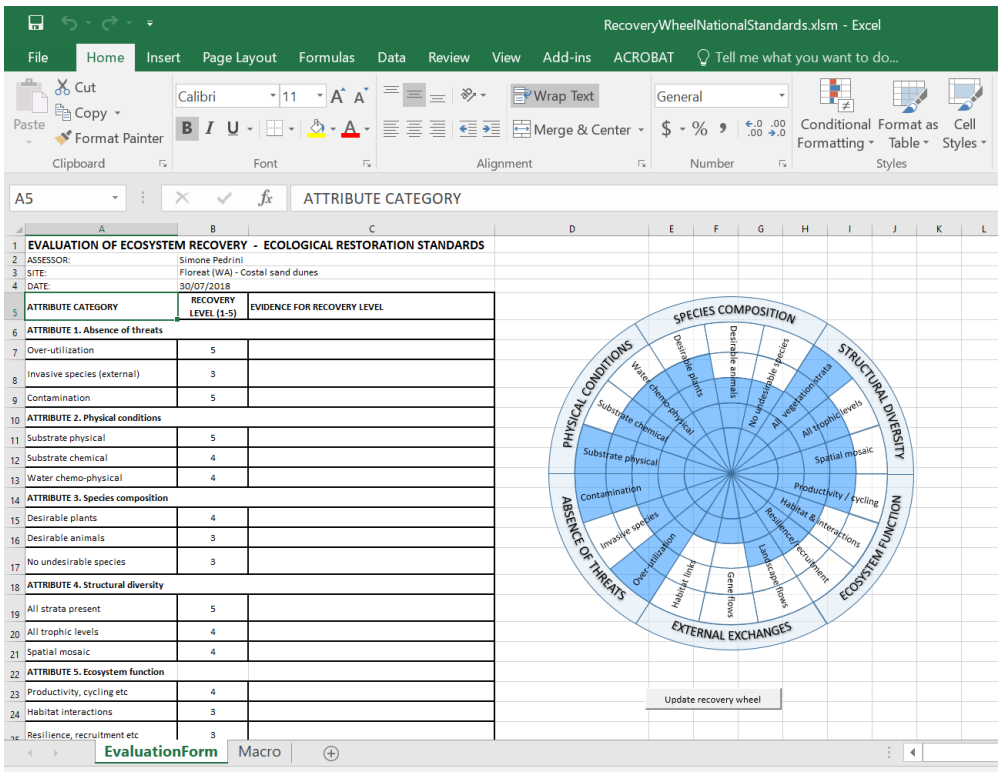
*Excel*

http://seraustralasia.com/standards/downloads/RecoveryWheelNationalStandards.xlsm

To make the recovery wheel more accessible and usable, I first developed an interactive excel spreadsheet. The file is separated into two pages. The first one (EvaluationForm) present both the pro-forma and recovery wheel, and the second page (Macro) contain the data and code necessary to run the interactive wheel. The primary user should use the page "EvaluationForm". By filling the cells in "recovery

level 1-5" for each attribute (18 in total) and then clicking on the "update recovery wheel" button, located underneath the wheel, the spokes of the recovery wheel would automatically be coloured (in blue) in accordance to the recovery level expressed in the table (Figure S2.1). For each attribute in the table, the operator could also provide a description of evidence for recovery.

This simple Excel tool was developed using macro and the Microsoft programming language Visual Basic (VBA). It is openly and freely available on the seraustralasia.org website, and it is also accessible via a link provided in the pdf version of the "2nd edition of the national standards". Due to its simplicity, it could be easily customised by practitioners whose restoration project need the evaluation of different attributes (e.g. Marine habitat restoration).




*Example of the excel based recovery wheel and table*

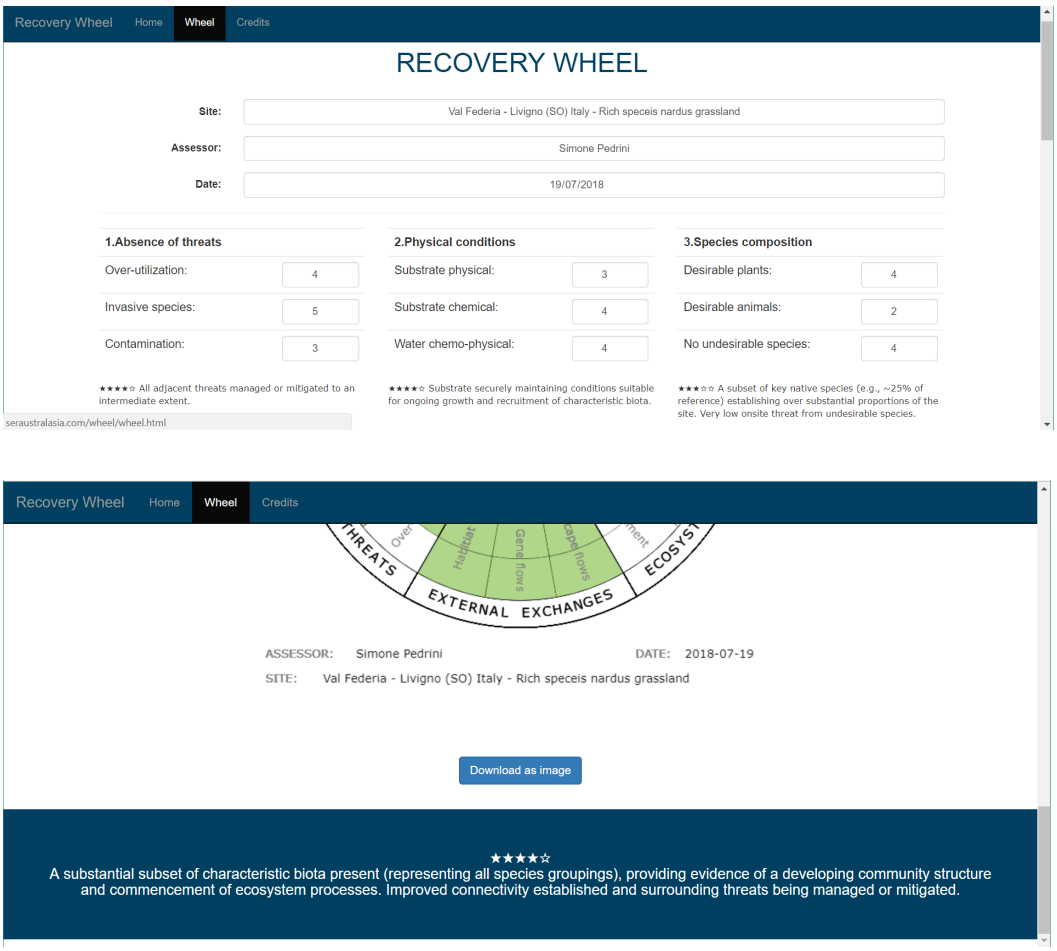
The excel based recovery wheel could be a useful tool during the restoration project planning and reporting phase when the operator has access to a computer. However, it is not practical when performing assessment/monitoring on the field, as excel could hardly be used on portable devices.

*Web-App*

http://seraustralasia.com/wheel/wheel.html

To solve this problem, I have worked on a web-based application, that would allow practitioners to access an interactive Pro-forma and recovery wheel online, through any browser.

This transition has posed several challenges in the programming phase, as the VBA language used for the excel version could not work online. The recovery wheel web application was therefore developed starting from a webpage written in HTML/CSS, hosted on the seraustralasia.com server. The shape of the wheel and the interactional behaviour between the data input table and the wheel was developed in Javascript.




*Details of the interactive Recovery wheel web application*

The web-based recovery wheel is composed of 3 web pages:

- The "home" page provides general information about the standards, a link to download the document and an introduction to the recovery wheel.
- The "wheel" page, host the interactive wheel application. The fill-in fields for general information (site, date and operator) are located at the top. The 18 attributes are grouped into six attribute categories, presented as separate blocks. Those blocks have been programmed in a bootstrap matrix template, so that are automatically re-arranged to fit the size of the screen better, allowing for smoother navigation and interaction from mobile devices. At the bottom of the page is located the recovery wheel. After filling out the general information and the attributes, and clicking on the "update wheel" button, the wheel is filled. An average star rating is automatically calculated and shown in the footer for all of the attributes, and for the attributes categories under each block. Along with the star rating, an interpretation of the rating is provided. Under the wheel, the button "download recovery wheel" allows obtaining a "jpg" image of the wheel and general information that could then be used in reports or presentations.
- The Credit page showed the logos and link to the organisation that have provided support for the development of the "recovery wheel web-app" and the "standards" and referenced the document of the "standards".

The main limitation of the web-app version is that it requires an online connection to be operational, but in many remote restoration sites, the internet is often unreliable or not available.

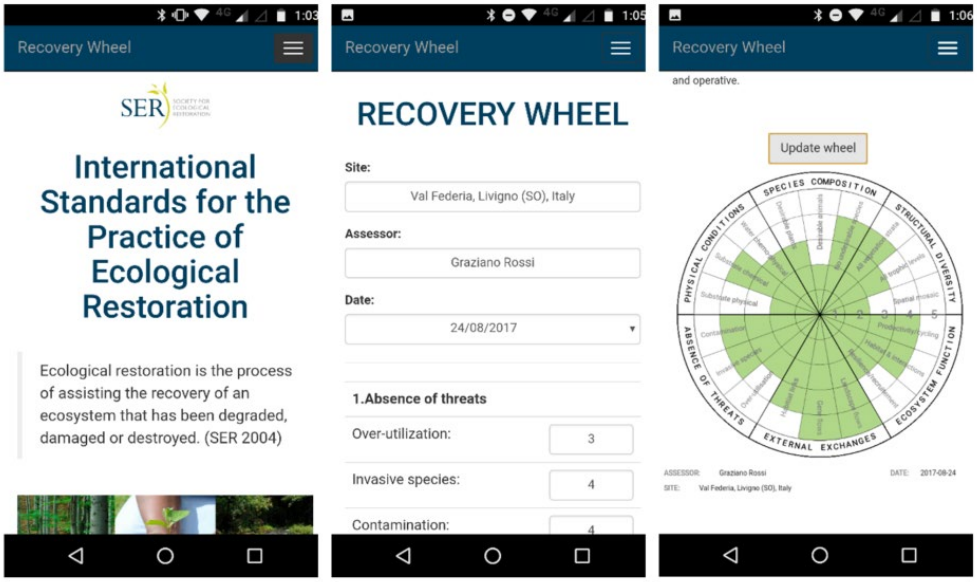
To solve this problem and further improve user interaction a mobile-app was then developed.

*Mobile-App*

The mobile-app is structured and operates precisely like the web-app. The HTML/CSS and javascript code were converted to Android (.apk) and IOS format (.ipa) with PhoneGap. The loading on the Android store was straightforward, and the mobile-app was available for download on the 23rd of August 2017. However, the process of obtaining the correct permission protocol from the IOS app store required a great deal of editing to the original code and further tinkering with the required developer certifications, delaying the publication of the app on the IOS store to November 21st 2017. The mobile app is accessible from both smartphones and tablets, once installed

does not require any internet connection to operate, and could, therefore, be used in the field. However, the save image functionality is not supported by the mobile-app version, and the best way for the user to save the updated wheel is with a screenshot. Both the IOS and Android versions are freely available for download at the following links:
https://play.google.com/store/apps/details?id=com.smnpedrini.recoveryWheel&hl=en

https://itunes.apple.com/us/app/recovery-wheel/id1315509658?mt=8




*Screenshots of the recovery wheel app on Android*

*Significance of the interactive recovery wheel*

Along with its use by practitioners, the web and mobile Recovery Wheel could be used for educational purposes. Used in the classroom and restoration site, will help the students understanding the basic concept of ecological restoration, and familiarising with its practice interactively and engagingly. It could also be introduced in the CERP (Certified Ecological Restoration Practitioners) program, run by SER, and offering the renewing or newly certified practitioners a useful tool to plan, manage and monitor their projects.

*Future development*

In its current form, the interactive Recovery Wheel offers some useful functionality but lacks the backend-database infrastructure that would allow for saving the data and information collected other than in the form of an image. Limited time and resources have so far allowed the development of the graphic interface and basic interactions. However, further investment and collaboration with database and native app programmer will be needed for the development of a complete restoration project management tool that could include, map integration, GPS coordinate from the site, upload of images, collection and storage of other relevant and useful information. This data could then be synchronised and uploaded to the cloud when an internet connection is available, allowing for the access, and use, of this management tool from multiple platforms. On the one hand this tool would help the practitioners running their projects, and on the other hand, allow SER and other funding bodies to have a standardised and up to date snapshot on how the global ecological restoration effort is progressing.

Extensive consultation with practitioners will be necessary, to develop an intuitive yet complete tool, which could address their planning, management and reporting needs.

### *S.2.4. International Network for Seed Based Restoration*

The International Network for Seed Based Restoration (INSR) is a thematic chapter of the Society for Ecological Restoration (SER) that brings together professionals, scientists, practitioners, students, industry, government and organisations from the international community who have an interest in promoting and enhancing seed-based solutions in restoration. Its foundation was originally discussed during the 2015 National Native Seed Conference in Santa Fe (New Mexico, USA). At the end of 2015, INSR was officially formed, and I developed its website www.ser-insr.org.

I had served as webmaster from the beginning, and I was elected for the role of Director at large at the end of 2016. I had then been re-elected for the same role in October 2018 and I will be serving in that position until 2021.

INSR currently (November 2018) has 386 members and 64 partner organisations from 28 countries. The website was reached by 5,696 unique visitors in 2017 and 6,015 in 2018 (Until November 26th). Since December 2015, 55 news articles have been published in the news section of the website http://ser-insr.org/news/. I have been involved in the process of editing and publishing the article online. I have directly contributed to the following articles:

- Pedrini S and Bhalsing K (2018) How to pellet seeds? http://ser-insr.org/news/2018/7/25/how-to-pellet-seeds
- Pedrini S (2018) Formation of the European native seed producers association. http://ser-insr.org/news/2018/5/26/formation-of-the-european-native-seed-producers-association
- Pedrini (2017) The native seed community shining at the global ecological restoration conference in Brazil. http://ser-insr.org/news/2017/9/7/the-global-native-seed-community-shining-at-the-global-ecological-restoration-conference-in-brazil
- Pedrini S (2017) Can seed coating boost seed based restoration outcome? http://ser-insr.org/news/2017/2/8/can-seed-coating-boost-seed-based-restoration-outcome

## S.3. Paper co-authorship statement

To Whom It May Concern

I, *Simone Pedrini*, contributed in *designing the study, developing the database for the organisation and analysis of the literature, interpreting the data, writing the manuscript and acting as corresponding author* to the paper/publication entitled *Seed Coating: Science or Marketing Spin? (2017) Trends Plant Sci 22:106–116.*
*doi: 10.1016/j.tplants.2016.11.002*

*(Signature of Candidate)*

I, as a Co-Author, endorse that this level of contribution by the candidate indicated above is appropriate.

*David Merritt:*

*Jason Stevens:*

*Kingsley Dixon:*

To Whom It May Concern

I, *Simone Pedrini*, contributed in *designing the study, developing and testing, the protocol development tool, interpreting the data, co-writing the manuscript and acting as corresponding author* to the paper/publication entitled *Protocol Development Tool (PDT) for seed encrusting and pelleting. (2018) Seed Sci Technol 46:393–405. doi: 10.15258/sst.2018.46.2.21*

*(Signature of Candidate)*

I, as a Co-Author, endorse that this level of contribution by the candidate indicated above is appropriate.

*Khiraj Balshing:* _____

*Adam Cross:* _____

*Kingsley Dixon:* _____

To Whom It May Concern

I, *Simone Pedrini*, contributed in *designing the study, performing the processing treatment and the experiments, interpreting the data, writing the manuscript and acting as corresponding author* to the paper/publication entitled *Optimizing seed processing techniques to improve germination and sowability of native grasses for ecological restoration. (2018) Plant Biol.*

*doi: 10.1111/plb.12885*

Simone Pedrini

*(Signature of Candidate)*

I, as a Co-Author, endorse that this level of contribution by the candidate indicated above is appropriate.

*Lewandrowski Wolfgang:* _____

*Jason Stevens:* _____

*Kingsley Dixon:* _____

# S.4. Copyright permission statement

12/17/2018 Rightslink® by Copyright Clearance Center

Copyright Clearance Center RightsLink®

Home | Account Info | Help

Title: Seed Coating: Science or Marketing Spin?
Author: Simone Pedrini, David J. Merritt, Jason Stevens, Kingsley Dixon
Publication: Trends in Plant Science
Publisher: Elsevier
Date: February 2017
© 2016 Elsevier Ltd. All rights reserved.

Logged in as: Simone Pedrini
Account #: 3001277634
LOGOUT

Please note that, as the author of this Elsevier article, you retain the right to include it in a thesis or dissertation, provided it is not published commercially. Permission is not required, but please ensure that you reference the journal as the original source. For more information on this and on your other retained rights, please visit: https://www.elsevier.com/about/our-business/policies/copyright#Author-rights

BACK | CLOSE WINDOW

Copyright © 2018 Copyright Clearance Center, Inc. All Rights Reserved. Privacy statement. Terms and Conditions.
Comments? We would like to hear from you. E-mail us at customercare@copyright.com

**Title:** Optimising seed processing techniques to improve germination and sowability of native grasses for ecological restoration

**Author:** S. Pedrini, W. Lewandrowski, J. C. Stevens, et al

**Publication:** Plant Biology

**Publisher:** John Wiley and Sons

**Date:** Aug 30, 2018





## Order Completed

Thank you for your order.

This Agreement between Mr. Simone Pedrini ("You") and John Wiley and Sons ("John Wiley and Sons") consists of your license details and the terms and conditions provided by John Wiley and Sons and Copyright Clearance Center.

Your confirmation email will contain your order number for future reference.

printable details

| | |
|---|---|
| License Number | 4491100213811 |
| License date | Dec 16, 2018 |
| Licensed Content Publisher | John Wiley and Sons |
| Licensed Content Publication | Plant Biology |
| Licensed Content Title | Optimising seed processing techniques to improve germination and sowability of native grasses for ecological restoration |
| Licensed Content Author | S. Pedrini, W. Lewandrowski, J. C. Stevens, et al |
| Licensed Content Date | Aug 30, 2018 |
| Licensed Content Volume | 0 |
| Licensed Content Issue | 0 |
| Licensed Content Pages | 10 |
| Type of use | Dissertation/Thesis |
| Requestor type | Author of this Wiley article |
| Format | Print and electronic |
| Portion | Full article |
| Will you be translating? | No |
| Title of your thesis / dissertation | SEED ENHANCEMENT RESEARCH FOR IMPROVING ECOLOGICAL RESTORATION |
| Expected completion date | Dec 2018 |
| Expected size (number of pages) | 120 |
| Requestor Location | Mr. Simone Pedrini<br>19 Callington Avenue<br><br>City Beach, WA 6015<br>Australia<br>Attn: Mr. Simone Pedrini |
| Publisher Tax ID | EU826007151 |
| Total | 0.00 AUD |