Native Perennial Grasses for Sustainable Pasture Systems

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Rehabilitation of degraded lands through the use of native grasses is likely to enhance productivity in poor performing agricultural and mining rehabilitation areas, thus improving industry sustainability by increasing biodiversity and restoring ecosystem function. At present, variable and unsuccessful native perennial pasture establishment is the result of (a) low availability of viable seed, (b) asynchronous and unreliable seed germination, (c) poor seedling emergence and (d) low seedling vigour. This project targets the development of a national program to deliver seed-based solutions to overcome seed germination and establishment barriers for a profitable and productive broad-acre native perennial grass pasture and restoration industry. Species of interest include: \textit{Austrodanthonia} sp., \textit{Bothriochloa macra}, \textit{Chloris truncata}, \textit{Dichanthium sericeum}, \textit{Enteropogon acicularis}, \textit{Microlaena stipoides} and \textit{Themeda triandra}. Innovative laboratory techniques were employed to address these barriers to successful native grass establishment.

It is envisaged that the developed seed-associated technology for these species of native perennial grasses will have a profound effects on Australian agricultural (pasture) and mining industries by: (a) improving restoration of degraded landscapes including mining and agricultural systems; (b) increasing efficacy of use of native seeds resulting in decreased costs and increased success of pasture establishment; (c) protecting and enhancing national native biodiversity and (d) developing knowledge and understanding of native seed biology.

An understanding of species specific germination biology has allowed significant improvements in germination performance of native grass seeds. The use of dormancy breaking treatments and germination stimulants has further improved germination performance of several species of native grass seed in laboratory environments. Translation of seed treatments to field conditions requires further research to establish the robustness of the technology however several seed treatments appear to be consistently improving germination across many species/accessions, these include seed priming with signalling compounds and burying seed to improve seed water relations.

This project was funded from RIRDC Core Funds which are provided by the Australian Government.

This report is an addition to RIRDC’s diverse range of over 1900 research publications and it forms part of our New Plants Products R&D program, which aims to facilitate the development of new industries based on plants or plant products that have commercial potential for Australia.

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\textbf{Craig Burns}
\textit{Acting Managing Director}
\textit{Rural Industries Research and Development Corporation}
Acknowledgments

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Abbreviations

A1108  Microlaena stipoides (Griffin) Harvest date: 03/08  
A2108  Microlaena stipoides (Griffin) Harvest date: 03/08  
B1108  Microlaena stipoides (Griffin) Harvest date: 03/08  
B1208  Microlaena stipoides (Griffin) Harvest date: 03/08  
Bod  Boddington (Perth Hills) Harvest date: 12/05  
C3  winter growing grass  
C4  summer growing grass  
D11a, b  Dichanthium sericeum - Department of Primary Industries, QLD, ecotype 11  
D16a, b  Dichanthium sericeum - Department of Primary Industries, QLD, ecotype 16  
Dc1  Austrodanthonia caespitosa - Cathy Waters line, Trangie NSW  
Dr1  Austrodanthonia racemosa – Monaro NSW  
DRfl  Austrodanthonia fulva – Monaro NSW  
GA  gibberellic acid (100 parts per million)  
H2O  water  
K  kinetin (0.05 mM)  
KAR1  karrikinolide (100 parts per billion)  
K20a, b  Themeda triandra - Department of Primary Industries, QLD, ecotype 20  
K24a, b  Themeda triandra - Department of Primary Industries, QLD, ecotype 24  
LiCl  lithium chloride  
Lig 183  Microlaena stipoides – Ko-warra Native Grasses, Euchla  
MPa  measurement of water potential in megapascals  
NaCl  sodium chloride  
NS281  Chloris ventricosa (Barwon) 02/2008 – Myrtleford (Native Seeds Pty Ltd)  
NS285  Chloris truncata (Common) 02/2008 – Myrtleford (Native Seeds Pty Ltd)  
NS289  Microlaena stipoides (Ovens) 03/2008 – Wangaratta (Native Seeds Pty Ltd)  
NS290  Microlaena stipoides (Bremmer) 03/2008 – Wangaratta (Native Seeds Pty Ltd)  
NS292  Microlaena stipoides (Griffin) 03/2008 – Wangaratta (Native Seeds Pty Ltd)  
NS418  Bothriochloa macra (Common) 03/2008 – Tamworth (Native Seeds Pty Ltd)  
NS449  Microlaena stipoides (Griffin) 12/2006 – Wangaratta (Native Seeds Pty Ltd)  
P EG  polyethylene glycol  
RH  relative humidity  
SA  salicylic acid (0.5 mM)  
SC204  Austrodanthonia caespitosa – Northam, WA 12/2006 (Sam Clarke)  
SC206  Austrodanthonia caespitosa – Kellerberrin, WA 12/2006 (Sam Clarke)  
SC210  Austrodanthonia sp. Goomalling – Quairading, WA 12/2006 (Sam Clarke)  
SC217  Chloris truncata – Grass Valley, WA 12/2006 (Sam Clarke)  
SC219  Austrodanthonia pilosa – Armadale, WA 12/2006 (Sam Clarke)  
SC222  Austrodanthonia setacea – Mundaring, WA 12/2006 (Sam Clarke)  
SUIN  surface sown, intact florets  
SUCL  surface sown, clean seeds  
SW  smoke water (1% solution)  
S8a  Heteropogon contortus - Department of Primary Industries, QLD, ecotype 8  
1cm IN  1cm sowing depth, intact florets  
1cm CL  1cm sowing depth, clean seeds  
Ψ  water potential
# Contents

Foreword ................................................................................................................................................ ii  
Acknowledgments .................................................................................................................................. iv  
Abbreviations ....................................................................................................................................... iv  
Executive Summary .............................................................................................................................. viii  
1. Introduction ...................................................................................................................................... 1  
2. Objectives ......................................................................................................................................... 2  
3. Materials and Methods .................................................................................................................... 3  
4. Results ............................................................................................................................................ 10  
5. Discussion ....................................................................................................................................... 29  
6. Implications .................................................................................................................................... 33  
7. References ....................................................................................................................................... 34
Tables

Table 1: Species used for trials, including cultivated species and wild collections................. 3
Table 2: Successful germination stimulating treatment results showing species tested, collection number/accession, seed age (A=aged >8 weeks, F=fresh<8 weeks), initial germination (%) without treatment, highest germination (%) achieved with corresponding treatments, floret (= minus floret; + = floret intact), heat (=no heat; + = heat 100°C 30 mins), stimulant (H2O = water; GA = gibberellic acid 100ppm; SW = smoke water 1%; Karrikinolide 100 ppb). .......................................................... 11

Figures

Figure 1: (a) Faxitron X-ray machine used to non-destructively identify filled florets (b) The Air separator Zigzag-1 machine used to separate filled (heavy) florets from non filled (light) florets and (c) an X-ray image of a *Microlaena stipoides* seed batch showing floret fill of the initial batch (left) and 100% floret fill (right) after separation using the Zigzag machine. ................................................................................................................. 4
Figure 2: (a) Field site preparation (b) replicate preparation, showing burial treatment and spacing and (c) Field site after sowing at DAFWA, South Perth WA. ................................................. 6
Figure 3: An example of pellet seed coating technology used for native grasses. ......................... 9
Figure 4: Photo showing experimental approach to test seed treatments under glasshouse conditions. ..................................................................................................................................... 12
Figure 5: Germination Rate Index (GRI) and Germination (%) results for glasshouse (left hand graphs) and field (right hand graphs) trials. .................................................................................. 13
Figure 6: Photos of the field trial highlighting (a) *M. stipoides* (Lig 183) (b) *A. caespitosa* (Dc1) and (c) *M. stipoides* (Lig 183) & *M. stipoides* (Griffin) 4 months after sowing .... 14
Figure 7: Germination Rate Index (GRI) and Germination (%) results for glasshouse (1) and field (2). (A) = *Chloris truncata*, (B) = *Dichanthium sericeum*. ............................................................ 16
Figure 8: Results from field trial highlighting presence of GA treated (a) *Chloris truncata* and (b) *Dichanthium sericeum* 2 months after sowing (c) *Chloris truncata* and (d) *Dichanthium sericeum* 4 months after sowing.......................................................... 17
Figure 9: Seed germination percentage as influenced by 0, 1, 2, 3 months after-ripening period. (a) = *Austrodanthonia setacea*, (b) = *Chloris truncata*, (c) = *Dichanthium sericeum*, (d) = *Themeda triandra*. ........................................................................................... 18
Figure 10: Germination (%) after 0, 3, 6 months storage in (a) *Austrodanthonia caespitosa* (Dc1) and after (b) *Microlaena stipoides* (Lig 183). .................................................................................. 19
Figure 11: Influence of water stress levels on percent seed germination in (a) *Austrodanthonia setacea*, (b) *Austrodanthonia caespitosa* (Dc1), (c) *Bothriochloa macra*, (d) *Chloris truncata*, (e) *Enteropogon acicularis*, (f) *Microlaena stipoides* (Lig 183)........ 21
Figure 12: Effect of stress tolerance compounds on seed germination under saline conditions in (a) *Austrodanthonia setacea*, (b) *Austrodanthonia caespitosa* (Dc1), (c) *Chloris truncata*, (d) *Microlaena stipoides* (Lig 183)................................. 22
Figure 13: Overcoming water stress using seed priming treatment combinations in (a) *Austrodanthonia setacea*, (b) *Austrodanthonia caespitosa* (Dc1), (c) *Chloris truncata*, (d) *Enteropogon acicularis*, (e) *Microlaena stipoides* (Lig 183). ........................................................................... 24
Figure 14: The influence of seed coating on germination under laboratory conditions in Intact (florets intact) or Clean (caryopses removed from florets) of (a) *Austrodanthonia caespitosa* (Bod 12/05), (b) *Austrodanthonia caespitosa* (Dc1), (c) *Chloris truncata*, (d) *Dichanthium sericeum*, (e) *Microlaena stipoides* (Lig 183). ............................................. 25

Figure 15: The influence of seed coating on germination under glasshouse conditions in Intact (florets intact) or Clean (caryopses removed from florets) of (a) *Austrodanthonia caespitosa* (Bod 12/05), (b) *Austrodanthonia caespitosa* (Dc1), (c) *Chloris truncata*, (d) *Dichanthium sericeum*, (e) *Microlaena stipoides* (Lig 183). ....................................... 27

Figure 16: The influence of seed coating on germination under field conditions in Intact (florets intact) or Clean (caryopses removed from florets) of (a) *Austrodanthonia caespitosa* (Bod 12/05), (b) *Austrodanthonia caespitosa* (Dc1), (c) *Chloris truncata*, (d) *Dichanthium sericeum*, (e) *Microlaena stipoides* (Lig 183). ...................................... 28
Executive Summary

What is the report about

The objective of this report is to provide information on the potential of seed germination enhancement treatments for native perennial grass species. By overcoming seed dormancy issues associated with native grasses and stimulating germination, it is anticipated that significant improvements can be made to establishment success under field conditions. This report focuses on, but is not limited to, four target species of native grass (Microlaena stipoides, Austrodanthonia caespitosa, Chloris truncata and Dichanthium sericeum).

Who the report is targeted at

This report is targeted at native grass seed end users (suppliers, growers and collectors), as well as researchers of native grasses. The content of this report has wide implications for the use of native grasses in land restoration and production systems.

Background

Mining and agricultural industries ($44 and $8 billion/pa export value) produce significant landscape alterations and loss of plant biodiversity, thus there is an urgent need to identify native perennial species with potential to be successful in these systems. Currently, low availability of viable seed and poor seedling establishment result in unsuccessful native perennial pasture production. By alleviating seed-based issues, land productivity will increase and restoration will become more likely.

Aims/objectives

The objective of this project is to address these problems through investigation of the best means to achieve a high establishment success via alleviating after-ripening, developing mechanisms to break seed dormancy, using seed coatings and seed priming.

Methods used

Seed quality issues (i.e. viability, germination rates, maximum germination percentage) were assessed in 17 species across 53 ecotypes collected from QLD, VIC and WA. Based on preliminary results many of these species possess physiological dormancy, which appears to be significantly influenced by seed covering structures (their removal increases germination). Many grass seeds exhibit after-ripening phenomena, which is strongly linked to storage conditions. We assessed the effects of post-harvest storage on germination optimising temperature/humidity to maximise the rate of after-ripening.

We also investigated priming seeds with germination promoters (including smoke water, the novel chemical isolated from smoke – Karrikinolide, and gibberellic acid), removal of covering structures and seed burial to enhance germination. These experiments were undertaken in multiple dormancy breaking studies in controlled environments.

Seed priming technology was used to alleviate slow and asynchronous germination. Seed priming with the addition of stress tolerance chemicals including the plant signalling compounds salicylic acid and kinetin was used to improve establishment success of species under a range of stressful conditions that limit seed germination (i.e. drought and high salt).

Seed coating technology was applied to florets and cleaned seeds of five species. The germination enhancing chemical gibberellic acid was incorporated into the polymer pellet and film coats to determine whether seed coating can improve germination rates and percentages in the field and improve seed delivery to site.
Treatment that delivered improved vigour without detrimental effects were transferred to the field. Field trials were established at the Department of Primary Industries Walkamin Research Station, Queensland and Victoria and Department of Food and Agriculture, South Perth WA and Shenton Park Research Facility WA. At each site treatments of accessions were planted into 100m² plots using a completely randomised design. Results are only presented for WA trials.

**Results/key findings**

Seed quality is a significant issue with native grasses, particularly if collected from wild stands. This project highlights two techniques (X-ray analysis and air separation) that provide rapid means to improve seed quality for end users.

An understanding of the species/accession-specific germination biology has allowed significant improvements in germination performance of native grass seeds. The use of dormancy-breaking treatments and germination stimulants has further improved germination performance of several species of native grass seed in laboratory environments, for example:

- *Austrodanthonia* species respond well to smoke water treatment at 1% solution, which increases germination from 26% to 96% if seeds are cleaned
- *Chloris truncata* germination can be increased in some ecotypes from 5% to 48% when treated with gibberellic acid and further increased to 86% if also heated at 100°C for 30 minutes
- this heat treatment will also increase germination of *Themeda triandra* seeds from 5% to over 30%
- Gibberellic acid enhances germination of *Dichanthium sericeum* from 9% to 74% if the florets remain intact; if the caryopses are removed >90% germination can be achieved without further treatment
- *Enteropogon acicularis* germination was improved from 48% to 99% by removing the caryopses from the enclosing floret structures without the need for germination enhancing chemicals
- *Microlaena stipoides* seeds germinate to between 90 and 100 % when fresh, either as intact florets or as cleaned seeds without germination stimulants but gibberellic acid may increase the rate of germination.

The use of stress signalling compounds appears to have a broader effect on native grass species, with salicylic acid and kinetin improving germination under water and salinity stress in many species. Response to drought stress was species specific however *M. stipoides* appears to be the most resilient native grass tested, at least at the germination phase.

After-ripening and storage treatments highlighted species differences. However when stored under ideal conditions (cool and dry), there appears to be no negative effect of storage in *A. caespitosa* and *M. stipoides* after 6 and 12 months respectively.

**Implications**

This research has direct implications for the native grass industry, particularly for suppliers and collectors of native seed. Relatively simple and cheap seed treatments produced significant improvements in native grass germination and were useful across many species/accessions tested. The approach used in this report can be readily adapted to industry to improve the quality of the end product. This quality increase will hopefully facilitate broad scale adoption of native grasses in Australia.
Recommendations

This study highlights the capacity to manipulate native grass seed germination under stressful conditions to improve germination success. Several key seed treatments have been identified as showing promise including, (a) seed cleaning (b) seed burial for favourable water relations and (c) seed priming through the use of smoke water, gibberellic acid, salicylic acid and kinetin. The translation to field establishment requires further research despite alleviation of some barriers to germination.
1. Introduction

Large areas of Australia’s land are ecologically degraded including those that have been cleared and are now subject to erosion, rising water tables, dryland salinity, weed invasions or other ecological problems. This indicates that current farming systems (high-nutrient-requiring annual crops and pastures with shallow root systems) are not sustainable (Lodge 1994). There is an urgent need to identify perennial species that have the potential to be successful in the low to medium rainfall (<300-500 mm) regions of Australia. Commercial pasture species in southern Australia have been imported from the Mediterranean basin, the basis being that such species are well adapted to both extreme climatic conditions and can withstand grazing pressure. However, Australia with its diversity of climate and growing conditions has a very rich native biodiversity of grass species, many of which were initially overlooked for agricultural value. Given the large genetic diversity which exists, there is huge potential for the development of native perennial species that are already well adapted to the climatic/edaphic conditions of Australian farming/pasture systems (Bennett et al. 2003).

1.1 Native Grass Species Attributes

Previous research on developing low input grasses useful in limiting environments (LIGULE) has identified native grass species with pasture potential (Johnston et al. 1999). Species were chosen from a range of attributes including persistence, vigour, productivity, palatability, morphology, and characteristics related to seed production (Mitchell et al. 2001). The LIGULE study determined that research was needed to develop methods of producing seed cheaply and efficiently.

Currently, low availability of viable seed, asynchronous and unreliable seed germination, poor seedling emergence and low seedling vigour result in variable and unsuccessful native perennial pasture production. This has resulted in large associated costs of seed production (Crosthwaite et al. 1996), unreliable establishment leading to high establishment costs (Vere et al. 2002) and limited broad-scale adoption of native perennial grasses. By addressing these fundamentally limiting factors through investigation of the best means to achieve high establishment success via alleviating after-ripening, breaking of seed dormancy mechanisms and use of the new innovations of seed priming and coating, it is anticipated that greater establishment of native perennial grasses at lower costs will inevitably result in a more successful native pasture industry.

1.2 Target Species

Native perennial grass species have been evaluated for use in agricultural pasture systems. Seventeen species have been identified which show great promise for Australian pasture systems in either northern or southern Australia (Table 1). Target species/genera were determined prior to project commencement based on careful coordination with perceived markets, identified by Native Seeds Pty Ltd, and research criteria requirements, developed by the CRC for Plant-Based Management of Dryland Salinity.

Suitable markets now exist for native perennial pastures that have naturally developed greater suitability to problem areas such as acidic soils, saline and waterlogging susceptible soils where use efficiency is an important consideration, low input farming systems (i.e. low nutrient), and areas susceptible to pests and disease.
2. Objectives

The aim of the project was to develop a national program to deliver seed-based solutions to overcome seed germination barriers for a profitable and productive broad-acre native perennial grass pasture and restoration industry. This aim will be met via the following objectives:

- refine new advances in seed production technology to increase commercial availability of native perennial grass species (including the use of the discovery by the applicant of one chemical in smoke that stimulates germination of native species) for pasture systems and rehabilitation sites

- define and prioritise the information and implement technology required to overcome barriers to commercial production, focussing on improving seed germination, seedling vigour and seedling stress tolerance

- increase the efficiency of seed-to-site establishment, by targeting a series of high potential native pasture species and recent innovations in seed technologies including polymer-based seed coatings for efficient delivery of germination enhancement and growth promoters.
3. Materials and Methods

3.1 Species Selection

Seventeen species have been identified which show great promise for Australian pasture systems in either northern or southern Australia (Table 1). Of these 17 only nine have been available for evaluation in this project. As previously mentioned, target species/genera were determined with input from Native Seeds Pty Ltd and the CRC for Plant-Based Management of Dryland Salinity. Other species have been collected from wild populations in the Western Australian wheat-belt region, six species from Victoria and one from Queensland.

Table 1: Species used for trials, including cultivated species and wild collections.

Collection locations include several populations from northern and southern Australia. Rows highlighted in grey indicate the species chosen for evaluation by Native Seeds Pty Ltd, and CRC for Plant-Based Management of Dryland Salinity.

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<tr>
<th>Species</th>
<th>Collecting No/ Accession</th>
<th>Collection Location</th>
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<td>Trangie NSW</td>
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<td>Austrodanthonia caespitosa</td>
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</tr>
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<td>Austrodanthonia richardsonii</td>
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<td>Austrodanthonia setacea</td>
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</table>
3.2 Seed Quality and Viability Assessment

Seed viability and/or seed fill was determined on every seed batch by low intensity, high-resolution x-ray analysis (Faxitron specimen radiography system). This indicated the number of florets that contain caryopses (Fig 1). The x-ray unit is designed for high resolution radiographic imaging of medical specimens, in particular, tissue and bone. This unit has shown to be extremely successful for use in seed analysis and allows for rapid and multi-sample determination of seed viability, non-destructive auditing of seed quality, non-invasive interrogation of internal seed morphology and real-time investigation of seed dormancy, embryo maturation and growth.

![Figure 1](a) Faxitron X-ray machine used to non-destructively identify filled florets (b) The Air separator Zigzag-1 machine used to separate filled (heavy) florets from non filled (light) florets and (c) an X-ray image of a *Microlaena stipoides* seed batch showing floret fill of the initial batch (left) and 100% floret fill (right) after separation using the Zigzag machine.
After the initial seed batch assessment, if the batch has been identified as containing empty or poor quality seed, it will be put through Air separator zigzag machinery to separate the empty (light) florets from the filled (heavy) florets, thus ensuring optimal quality and seed fill (Fig 1) for use in experimental trials.

3.3 Germination Enhancement

3.3.1 Germination stimulating treatments

All species were tested for initial germination to identify the presence of seed dormancy mechanisms and alleviation requirements. Treatments examined the impacts of surrounding structures on germination/dormancy behaviour (intact florets vs. hand clean seed), heat shock to overcome after-ripening (no heat vs. seed heated at 100°C for 30 minutes) (Tieu et al. 2001), application of germination stimulants including gibberellic acid (100 ppm), smoke water (1%) and karrikinolide (KAR1) (100 ppb), all known to stimulate germination and/or alleviate physiological dormancy (Dixon et al. 1995, Flematti et al. 2004), a phenomena common to the Poaceae. For germination stimulant experiments, all seeds were soaked in treatments for 24 h before plating onto 82 mm diameter glass microfibre filter paper in 90 mm plastic Petri dishes soaked with tissue culture grade water. There were four replicates of 25 seeds per treatment. Petri dishes were sealed with parafilm and incubated at 20°C in the dark. All plates were scored for germination at 7, 14, 21 and 28 days.

3.3.2 Statistical analysis

Data was analysed using SigmaStat statistical analysis software. Germination stimulating treatments were analysed using ANOVA. Percentage values were arc-sine transformed prior to analysis. Significance in the results section refers to a difference at the 5% level ($P < 0.05$).

3.4 Field Trials

3.4.1 Germination stimulating treatments

The aim of this experiment was to observe if germination enhancement of grasses obtained in the laboratory could be readily transferred to soil and field conditions. Germination treatments (as described above, section 3.3) were applied to two accessions of $M. stipoides$ (Griffin and Ligule 183) and one accession of $A. caespitosa$ (De1), $C. truncata$ and $D. sericeum$. Both $M. stipoides$ lines were tested with control, $H_2O$ and GA only as previous trials indicated that these treatments were the most effective for germination. For $A. caespitosa$, $Chloris truncata$ and $Dichanthium sericeum$ seeds were tested with all priming treatments.

As field trials for C3 species were conducted in winter (average minimum temperature 7.7°C, maximum temperature 18.3°C) the laboratory trial previously tested was repeated except, all plates were incubated at 18/7°C (12h light/12h dark). As field trials for C4 species were conducted in spring (average minimum temperature 13.5°C, maximum temperature 24.2°C) the laboratory trial previously tested was repeated except, all plates were incubated at 26/13°C (12h light/12h dark). This incubation temperature was chosen as it closely replicated the temperatures experienced in the field at the time of the trial.

3.4.2 Germination and emergence trials

Glasshouse trials were conducted during autumn/winter at Kings Park and Botanic Garden. All seeds were soaked in priming treatments (as described above) for 24 h then dried for two days before sowing. Four replicates of each treatment were sown into punnets filled with silica sand (30 seeds per punnet). Punnets were watered daily and emergence was monitored every 3 days for 14 days, then at 21 and 28 days.
Field trials were established concurrently with laboratory and glasshouse trials at the Department of Agriculture and Food Western Australia (DAFWA), Perth Western Australia. The site was characterised by a sandy-loam soil type, with irrigation occurring every other day. Seed enhancement treatments were the same as above, i.e. primed for 24 h and dried back to initial water contents over two days. Each treatment consisted of 50 seeds planted in a row of 40 cm, and was replicated 4 times (Fig 2). A template press was used to accurately mark out replicate lines and bury seeds to exactly 1cm (Fig 2b, c). Emergence was monitored every 3 days for 14 days, then at 21 and 28 days. Fresh seeds of three accessions of *Chloris truncata* and two accessions of *Austrodanthonia caespitosa* were collected from the wheatbelt area of Western Australia and used for the initial germination trials. Also, two accessions of *Microlaena stipoides* from Victoria (Griffin and Ligule 183 lines), four accessions of *Dichanthium sericeum* and four accessions of *Themeda triandra* from Walkamin, Queensland and one accession of *Austrodanthonia caespitosa* (De1 line) from Victoria were used. The C3 trial was established in winter and the C4 trial was established in spring (see 3.4.1). Field trials in Victoria are not presented due to the unavoidable compromisation of these trials brought about by restrictions in water allocations and the drought experienced over the duration of this project.

### 3.4.3 Statistical analysis

Data was analysed using SigmaStat statistical analysis software. Germination stimulating treatments were analysed using a 2-way ANOVA design, and the glasshouse and field trials were analysed using a 3-way ANOVA design. Percentage values were arc-sine transformed prior to analysis. Significance in the results section refers to a difference at the 5% level \( P < 0.05 \).

![Figure 2:](image)

(a) Field site preparation (b) replicate preparation, showing burial treatment and spacing and (c) Field site after sowing at DAFWA, South Perth WA.
3.5 After-ripening

3.5.1 After-ripening treatments

Freshly harvested native grass seeds usually have at least some level of dormancy, which is released over time, known as after-ripening (Hagon 1976). After-ripening is a period of usually several months of dry storage at room temperature of freshly harvested, mature seeds and is a common method used to release dormancy and to promote germination (Bewley and Black, 1992).

In this experiment fresh seeds of *Austrodanthonia setacea, Chloris truncata, Dichanthium sericeum* and *Themeda triandra* were placed into accelerated after-ripening conditions (50% relative humidity, 45°C) to establish whether these species have after-ripening requirements for successful germination. 50% RH was chosen to imitate ambient humidity. Due to a lack of seeds available for experimental purposes it was only possible to test one RH% and one temperature.

Intact florets were placed into an environment of 50% relative humidity at 20°C to allow the seeds to equilibrate for 4 weeks. Seeds were then double sealed in foil bags and placed at 45°C (for the duration of the experiment).

Seeds were removed from the after-ripening environment at 1, 2 and 3 months and primed in either water (control), gibberellic acid (100 ppm) or smoke water (1%) for 24 hours. After priming, 25 seeds were placed onto glass filter papers moistened with tissue culture grade water in Petri dishes in replicates of four, except *Themeda triandra* where three replicates of 20 seeds were used due to a shortage of seeds. Petri dishes were sealed with plastic cling wrap and placed into an incubation chamber at 26/13°C (12 hours light and 12 hours dark). Germination was scored every 7 days for 4 weeks.

3.5.2 Statistical analysis

Data was analysed using a 2-way ANOVA design with SigmaStat statistical analysis software. Percentage values were arc-sine transformed prior to analysis. Significance in the results section refers to a difference at the 5% level ($P < 0.05$).

3.6 Seed Storage – the effect of temperature and relative humidity

3.6.1 Relative Humidity

Storage trials were carried out over 12 months on seed lots with two different moisture contents. Seeds were placed in relative humidity cabinets at either 20% (dry conditions) or 50% (ambient conditions) humidity using non-saturated lithium chloride solutions.

Lithium chloride (LiCl) solutions were prepared by adding 640g of LiCl to 1 L of water for the 20% RH and 370g LiCl to 1 L of water for the 50% RH. Solutions were prepared in plastic electrical boxes, tightly sealed and allowed to equilibrate for 24 hours at constant 20°C before adding the seeds.

3.6.2 Storage Temperatures

To investigate the influence of the storage environment on seed longevity, seeds were stored at three temperatures (23°C, 5°C and -18°C) and two seed water contents (20% and 50% relative humidity) as prepared above.

After equilibration for four weeks, seeds were removed from the humidity cabinets, double sealed in foil bags and placed in 23°C (room temperature conditions), 5°C (cold storage conditions) and -18°C (freezer conditions). Seeds were then removed at intervals of 3, 6 and 12 months to determine germination potential in order to monitor seed quality over time.
After storage, 25 randomly selected seeds were placed onto glass filter papers moistened with tissue culture grade water in Petri dishes in replicates of four. Petri dishes were sealed with plastic cling wrap and placed into an incubation chamber at 20°C for 12 hours light and 12 hours dark. Germination was scored every 7 days for 4 weeks.

### 3.6.3 Statistical analysis

Data was analysed using a 2-way ANOVA design with SigmaStat statistical analysis software. Percentage values were arc-sine transformed prior to analysis. Significance in the results section refers to a difference at the 5% level ($P < 0.05$).

### 3.7 Stress Tolerance

#### 3.7.1 Water stress – germination at different water potentials

The effect of water potential on *A. setacea*, *A. caespitosa* (Dc1), *B. macra*, *C. truncata*, *E. acicularis* and *M. stipoides* (Lig 183) was investigated by supplementing germination media with either polyethylene glycol (PEG8000) or sodium chloride (NaCl). PEG8000 and NaCl are osmolytes that are commonly used to control water potential in seed germination studies (i.e. controls the ability of seed to take up moisture). Seeds can be tested over a range of osmotic potentials (where 0 equates to soil field capacity and a more negative values (i.e. -1.5 MPa) equates to “drier” conditions). Osmolytes were added to germination paper equating to iso-osmotic potentials of 0, -0.25, -0.5, -0.75, -1 or -1.5 MPa. The osmotic potentials of PEG8000 or NaCl were calculated by equations 1 and 2, respectively, where $x$ is the concentration of PEG8000 by %w/v (Michel and Kaufmann 1973) or the concentration of NaCl (mM).

$$\psi_{\text{PEG}} \text{ (MPa)} = -7.6049x^2 - 33.025x + 4.83$$  
(1)

$$\psi_{\text{NaCl}} \text{ (MPa)} = -0.0045x - 0.0218 \quad (R^2 = 0.9981)$$  
(2)

#### 3.7.2 Germination under saline conditions

To improve germination vigour of *A. setacea*, *A. caespitosa* (Dc1), *C. truncata* and *M. stipoides* (Lig 183) under three saline conditions (0, -0.5, or -1 MPa NaCl), the plant signalling compounds: 100ppm gibberellic acid, 0.05mM kinetin (K) (Khan *et al.* 2003), and 0.5mM salicylic acid (SA) (Senaratna *et al.* 2003) were examined. Salicylic acid was dissolved in 1 mL of 100% ethanol prior to adding to 1L of water (Williams *et al.* 2003). Four replicates of 25 seeds with florets removed were placed on germination papers moistened with one of the plant signalling compound/NaCl combinations. Petri dishes were sealed with plastic cling wrap and placed into an incubation chamber at 20°C for 12 hours light and 12 hours dark. Germination was scored every 7 days for 4 weeks.

#### 3.7.3 Germination under water stress using plant signalling compounds

Treatments that resulted in the most significant improvement in germination under NaCl-induced water stress identified above were subsequently assessed as potential seed-priming agents. 100 ppm GA3, 0.05mM K, 0.005mM SA, GA3 + K, GA + SA, K + SA, GA + K + SA combinations were used to test germination of *A. setacea*, *A. caespitosa* (Dc1), *C. truncata*, *E. acicularis* and *M. stipoides* (Lig 183) under water stress (PEG8000 solutions with iso-osmotic potentials of 0 and -0.5 MPa). Four replicates of 25 seeds with florets removed were soaked in the seed-priming agents for 18 hours prior to plating on germination papers soaked in the PEG8000 solutions. Petri dishes were sealed with plastic cling wrap and placed into an incubation chamber at 20°C for 12 hours light and 12 hours dark. Germination was scored every 7 days for 4 weeks.
3.7.4 Statistical analysis

Data was analysed using a 2-way ANOVA design with SigmaStat statistical analysis software. Percentage values were arc-sine transformed prior to analysis. Significance in the results section refers to a difference at the 5% level ($P < 0.05$).

3.8 Seed coating technology

3.8.1 Seed coating methodology

Improved seed coating technology has been used in recent years in a range of industries, including agriculture, horticulture, turf production and floriculture. It has the ability to impact on crop yield, water use efficiency, improved germination synchronisation, final germination percentages and plant health and disease resistance.

Pellet coating is used to create a smooth, uniformly shaped pellet to improve seed flow through machinery and enable accurate delivery of seed to site. Pellets are particularly useful in fluffy grass florets where coating is applied to smooth the floret, prevent clumping and apply germination enhancing chemicals, fungicides and/or identification colourants. Film-coating is used to apply the same additives as pellets in a light film without changing the shape of the seed or floret. The pellet or film coat is produced by mixing a binder (polymer), colour, water and desired chemical (if necessary) and applying this to either the florets or cleaned seeds through specially designed machinery.

In this project we coated intact florets and cleaned seeds of *A. caespitosa* (Bod 12/05), *A. caespitosa* (Dc1), *C. truncata*, *D. sericeum* and *M. stipoides* (Lig 183) with either pellet- or film-coats. Water or GA$_3$ (1000ppm) were added to the coats to determine whether seed coat delivery of GA$_3$ improves germination synchronisation and percentages. Film coating was applied to intact florets and cleaned seeds while pellets coating (Fig 3) was applied to cleaned seeds only due to a lack of available seeds (a 50g minimum requirement for intact florets). Coating was provided by Seed Solutions Pty Ltd.

Seed coats were tested under laboratory, glasshouse and field conditions as per section 3.4.

3.8.2 Statistical analysis

Data was analysed using ANOVA with SigmaStat statistical analysis software. Percentage values were arc-sine transformed prior to analysis. Significance in the results section refers to a difference at the 5% level ($P < 0.05$).

![Image of a seed coated with a film coat.](image.jpg)

Figure 3: An example of pellet seed coating technology used for native grasses.
4. Results

4.1 Germination Enhancement – laboratory conditions

The germination response of species to seed cleaning, heat and germination enhancement treatments varied within and among species, with overall germination ranging from 0 to 100% (Table 2). Thirty-eight ecotypes showed significant responses to various germination enhancement treatments with gibberellic acid being the most influential. Overall 39 ecotypes had germination greater than 80% with a best bet germination treatment and only 12 had less than 80% final germination.

Germination was significantly improved in at least one of the germination treatments tested in four out of the five major grass species including *A. caespitosa*, *C. truncata*, *D. sericeum* and *T. triandra*. Removing *A. caespitosa* caryopses from florets improved germination performance (greater than 30% observed across all accessions tested). Heat treating seeds of *A. caespitosa* significantly decreased final germination percentage and was evident across all accessions tested (P<0.001). Subsequent treatment of *A. caespitosa* seeds with germination stimulants either had no effect (Dc1) (P=0.404) or significantly improved (WA collections) germination. For WA accessions, germination was significantly higher when clean seeds were treated with smoke water (P=<0.001) with an improvement of up to 96% being observed (Table 2).

Cleaning seeds of *C. truncata* significantly improved germination (P<0.001). Heat treating seeds also improved germination up to 31% (P<0.001). A significant interaction between gibberellic acid and heat was detected (P=0.009) improving germination to 86% (Table 2). *Dichanthium sericeum* germination was significantly higher when seeds were cleaned (P<0.001) and/or treated with gibberellic acid (P=0.006) compared to other treatments. Germination was improved up to 99% compared to the control (intact, not heat treated and germinated in water). Heat had no significant effect on germination performance of *D. sericeum* (P=0.641) (Table 2). *Themeda triandra* germination was poor in all treatments but higher in heat treated seeds (20-30%) compared to the non-heated seeds (<10%) (P<0.001). There was not a significant difference between stimulating treatments (P=0.134) or significant interactions between heat and treatment (P=0.890) but germination was between 2-10% higher in the smoke water treatment (Table 2). Germination of intact florets of both *M. stipoides* accessions was initially very high (>90%) with no significant improvement being made with seed cleaning (P>0.05). GA improved germination from 85% to 98% in intact florets (Table 2). An interesting trend was that the C4 grasses (*B. macra*, four ecotypes of *C. truncata* and *T. triandra*) all benefited from heat treatment (Table 2).
Table 2: Successful germination stimulating treatment results showing species tested, collection number/accession, seed age (A=aged >8 weeks, F=fresh<8 weeks), initial germination (%) without treatment, highest germination (%) achieved with corresponding treatments, floret (- = minus floret; + = floret intact), heat (- = no heat; + =heat 100°C 30 mins), stimulant (H20 = water; GA = gibberellic acid 100ppm; SW = smoke water 1%; Karrikinolide 100 ppb).

Grey shading of rows indicates the species selected for evaluation.

<table>
<thead>
<tr>
<th>Species Collecting No/ Accession</th>
<th>Seed Age</th>
<th>Initial %G</th>
<th>Highest %G</th>
<th>Floret</th>
<th>Heat</th>
<th>Stimulant</th>
</tr>
</thead>
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<td>SW</td>
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<td>CL</td>
<td>-</td>
<td>H2O,GA,SW,KAR</td>
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<td>H2O</td>
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4.2 Germination Enhancement – from laboratory to field

4.2.1 C3 Trial

*A. caespitosa* germination under laboratory conditions was significantly higher (P<0.001) in cleaned seeds (≥90%) compared to intact florets (30-60%) after 14 days. Priming treatment had no significant influence on seed germination (P=0.610). For *M. stipoides*, laboratory trials showed that removing caryopsis from intact florets significantly improved germination in both Griffin and Ligule 183 lines with germination improvement up to 20% and 60% respectively. Priming treatment had no significant influence on seed germination (P>0.05).

A significant improvement in seedling emergence from soil was made for *A. caespitosa* (Fig 5a) and both accessions of *M. stipoides* (Fig 5b, c). Under glasshouse conditions (Fig 4) the rate of *A. caespitosa* germination was increased up to 5%d⁻¹ by cleaning seed and priming with either GA or KAR₁ compared to the control (intact, surface, not primed). Burying seed appeared to negate the positive effect of seed priming on germination rate. The increase in germination rate of clean surface sown *A. caespitosa* seeds was accompanied by a high final percentage emergence, with 80% final germination in the GA treated seed compared to the control of 60%.

Under glasshouse conditions the rate of both accessions of *M. stipoides* was increased in surface sown seeds (Fig 5). Gibberellic acid further improved the germination rate of intact and clean seeds on the surface. Similarly, final germination was increased by 10% in *M. stipoides* (Lig 183). Buried seed germination varied between treatments in both *M. stipoides* accessions with water primed seed germination rate and final percentage higher in Ligule 183 compared to other treatments (Fig 5c).

Significant improvements were also observed in emergence of target species under field conditions (Fig 6). Overall buried seeds performed significantly better across all species, with surface sown seeds having a lower final germination percentage and a slower germination rate (Fig 5). In buried seeds, priming in any treatment (i.e. H2O, GA, SW, KAR₁) further improved *A. caespitosa* germination up to 5%d⁻¹. However, the best germination treatment appeared to be GA as this had the highest accompanying final percentage emergence of 54% (Fig 5a) compared to the control of 30%.

Germination and emergence of *M. stipoides* under field conditions was improved with gibberellic acid priming in the Griffin line with final germination improving by (>20%) (Fig 5b). Ligule 183 showed germination of 40-60% can be achieved by cleaning seed and burying at 1cm without applying stimulating treatments. In intact buried seeds, priming with H2O or GA can improve germination by 20% (Fig 5c).

![Figure 4: Photo showing experimental approach to test seed treatments under glasshouse conditions.](image)

(a) showing *M. stipoides* germination of intact seed (left) and cleaned seed (right) emerging from 1 cm depth (b) showing *A. caespitosa* germination of intact seed (left) and cleaned seed (right) emerging from 1 cm depth.
Figure 5: Germination Rate Index (GRI) and Germination (%) results for glasshouse (left hand graphs) and field (right hand graphs) trials.

(a) = *Austrodanthonia caespitosa* (Dc1), (b) = *Microlaena stipoides* (Griffin), (c) = *M. stipoides* (Ligule 183). Surface = surface sown, 1cm = seeds/florets sown at 1cm depth. Intact = florets intact, Clean = caryopses removed from florets. GRI indicated by bar columns and germination % indicated by scatter plots; black = not primed, white = water primed, grey = gibberellic acid primed, black with white dots (or x for germination) = smoke water primed, white with black dots (or triangle for germination) = karrikinolide primed.

Although not part of the experiment, observations made after the summer period following the field trial highlighted that plants of *C. truncata* and *D. sericeum* derived from the GA treatment persisted to a much higher level than non-GA treated seeds (Fig 8).
Figure 6: Photos of the field trial highlighting (a) *M. stipoides* (Lig 183) (b) *A. caespitosa* (Dc1) and (c) *M. stipoides* (Lig 183) & *M. stipoides* (Griffin) 4 months after sowing.

### 4.2.2 C4 Trial

*Chloris truncata* germination under laboratory conditions was significantly higher (P<0.001) in cleaned seeds (>90%) compared to intact florets (<15%) after 14 days. Heat treated seeds showed significantly less germination (P=0.036) compared to unheated seeds. Priming treatments had no significant influence on seed germination (P=0.993).

For *Dichanthium sericeum*, laboratory trials showed that removing caryopsis from intact florets did not improve germination (P=0.575). Similarly, heating seeds did not show a significant improvement in germination when compared to non-heated seeds (P=0.271). Germination was higher in water primed seeds compared to KAR$_1$ (P<0.001) and smoke water (P=0.10) treated seeds and higher in gibberellic acid primed seeds compared to karrkinolide (P<0.001).

A significant improvement in seedling emergence from soil was made for *C. truncata* (Fig 7). Under glasshouse conditions the rate of *C. truncata* germination was increased 20-fold when surface sown compared to 1 cm sown. Cleaning seed improved germination rate across all treatments (P=0.008), as did, priming with either GA or KAR$_1$ when compared to the control (intact, surface, not primed) (P=0.005; P=0.028). Heating seeds prior to burial did not improve germination, nor was it detrimental (0.414). The increase in germination rate of surface sown *C. truncata* seeds was accompanied by a high final percentage emergence, with an improvement of up to 40% (Fig 7, 1A).

Sowing depth did not influence germination or emergence rates in *C. truncata* (P=0.166) under field conditions. Germination rate and final percentage was significantly lower in intact florets compared to clean seeds (P<0.001) when the seeds were heat treated. Priming seeds with gibberellic acid improved germination percentage (P<0.001) under certain situations compared to other treatments (P<0.001) however the best treatment was priming cleaned heat treated seeds in KAR$_1$ prior to burial (~10%
emergence increase and 2 fold germination rate increase). Heating seeds did not improve germination percentage (P=0.733) or speed of germination (P=0.688) (Fig 7, 2A). Overall field emergence was significantly lower than under glasshouse conditions (P<0.05).

Significant improvements were also observed in emergence of *D. sericeum* under glasshouse conditions. Intact seeds germinated faster and to a higher level than cleaned seeds (P<0.001). Germination rate was further improved with gibberellic acid however this was not consistent across treatments (Fig 7, 1B). Gibberellic acid was consistently the best treatment for final germination percentage across all treatments, with up to 80% germination being observed. Heat treating seeds increased the speed of germination (P=0.009) with further increases after KAR1 priming (P=0.036) (Fig 7, 1B). Surface sowing or heat treating *D. sericeum* seeds had no effect on germination rate index and final percentage emergence (Fig 7, 1B).

Germination and emergence of *D. sericeum* under field conditions was improved when seeds remained in florets (P<0.001) and treated with gibberellic acid (P<0.001) with germination between 10 to 15% higher compared to all other treatments (Fig 5, 2B). Seeds treated with gibberellic acid also showed a germination rate 5 to 12 times higher in intact florets sown at 1cm depth (Fig 9b). Heat treating seeds did not significantly improve germination rate (P=0.709) or germination percentage (P=0.891). The highest germination percentage was observed in non heat treated GA primed intact florets sown at 1cm depth (~70%) (Fig 7, 2B) compared to 80% in the glasshouse (Fig 7, 1B).
Figure 7: Germination Rate Index (GRI) and Germination (%) results for glasshouse (1) and field (2). (A) = *Chloris truncata*, (B) = *Dichanthium sericeum*.

Surface = surface sown, 1cm = seeds/florets sown at 1cm depth. IN = florets intact, CL = caryopses removed from florets. GRI indicated by bar columns; black = not primed, white with black dots = water primed, white = gibberellic acid 100ppm
primed, white with black stripes = smoke water 1% primed, grey = karrikinolide 100 ppb primed. Germination % indicated by scatter plots; triangle = not primed, square = water primed, black dot = gibberellic acid 100 ppm primed, grey dot = smoke water 1% primed, X = karrikinolide primed.

Figure 8: Results from field trial highlighting presence of GA treated (a) *Chloris truncata* and (b) *Dichanthium sericeum* 2 months after sowing (c) *Chloris truncata* and (d) *Dichanthium sericeum* 4 months after sowing.

Non GA treated seeds failed to persist.

### 4.3 After-ripening

All four species were at least partially dormant at the beginning of the experiment (<60% germination) (Fig 9). Fresh seeds of *A. setacea* germinated to 53% with a significant increase to 71% when primed with gibberellic acid (P=0.006). After one month in after-ripening conditions, germination was slightly decreased in the control (water) and gibberellic acid treatments but significantly higher (at 75% ) when treated with smoke water (P=0.029). Germination after two months was substantially less compared to the previous months with the water treated seeds only germinating up to 7%, and 24% and 19% for gibberellic acid and smoke water, respectively. Germination after three months after-ripening was zero (Fig 9a).

Dormancy of *C. truncata* seemed to be overcome after one and two months after-ripening where germination was significantly higher compared to time zero (P<0.001). After three months, however, germination was less than 1% (P<0.001) (Fig 9b). Smoke water consistently improved germination in the initial stages of after ripening. Poor initial germination of 27-33% in *D. sericeum* was not improved by after-ripening seeds or treating with germination enhancement treatments (P=0.081). The
total germination percentage was reduced after one and two months with no germination occurring after three months (Fig 9c). Germination of \textit{T. triandra} was less than 8\% across all after-ripening periods and treatments, with no germination occurring after three months (Fig 9d).

![Figure 9](image)

**Figure 9**: Seed germination percentage as influenced by 0, 1, 2, 3 months after-ripening period. (a) = \textit{Austrodanthonia setacea}, (b) = \textit{Chloris truncata}, (c) = \textit{Dichanthium sericeum}, (d) = \textit{Themeda triandra}.

Germination \% indicated by bar column; black = water only, light grey = gibberellic acid 100ppm treatment, dark grey = smoke water 1\% treatment.

### 4.4 Seed Storage – the effect of temperature and relative humidity

Storage of \textit{A. caespitosa} (Dc1) seeds was not detrimental to successful germination after six months storage at 20\% and 50\% relative humidity (P=0.096). Intact germination was significantly lower than cleaned seeds (P<0.001) across all treatments. However, intact seeds appeared to benefit from storage at 50\%, -18\degree C and 5\degree C and germination improved when compared to storage at three months at ambient (23\degree C) (P=0.038). After 6 months storage, clean seeds stored in dry conditions (20\% RH) showed a significant decline in germination compared to storage results at three months (P=0.020) (Fig 10a).

Germination of \textit{M. stipoides} remained high (>80\%) over the 12 month storage period in both intact florets and cleaned seeds. There was no difference in final germination percentages in the type of floret (P=0.106), relative humidity (P=0.094) or storage temperature (P=0.121). Germination was reduced, however, in cleaned seeds after 12 months storage at 23\degree C compared to the other storage temperatures (P<0.001) (Fig 10b). This may indicate that the viability of cleaned seeds may decline faster at warmer temperatures over time.
4.5 Stress Tolerance – germination at different water potentials

4.5.1 Water Stress

Cleaned *A. setacea* seeds have the ability to take up water more efficiently than seeds that remain in florets (Fig 11a). Germination of seeds remaining within the floret was up to 38% when water was not limited (control), but when seeds were removed from the florets (clean) germination reached 90% in control seeds and were still able to germinate in moderately dry (i.e. -0.75 MPa) conditions. Germination was less than 10% when water stress was high (i.e. -1.0 MPa) with no germination after this point.

Unlike *A. setacea*, *A. caespitosa* (Dc1) was able to withstand lower water potentials when seeds remained in the florets, particularly under ionic stress (NaCl) at -0.5 MPa (P<0.001) and when water availability was extremely limited i.e. -0.75 MPa (P<0.001) and -1.0 MPa (P<0.001) (Fig 11b).

There were significant differences in germination of *B. macra* seeds under osmotic (PEG) and ionic (NaCl) stress (P=0.002). Under osmotic stress seeds germinated more successfully when cleaned (P<0.001) but under ionic stress germinated better, even when water was only moderately limiting (-0.25 MPa) (P<0.001) when seeds remained within the florets (Fig 11c).

Final germination percentages were significantly different between intact florets and cleaned seeds (P<0.001) in *C. truncata*. Germination was more successful under osmotic conditions (P=0.047) when seeds remained within the florets but overall germination was higher in both osmotic and ionic
conditions when seeds were cleaned. Compared to the clean control seeds, however, germination was low (i.e. <40%) when water potentials were less than -0.25 MPa (Fig 11d).

Germination of intact *E. acicularis* seeds was less than 40% in the control seeds and less than 20% under low water stress conditions, which is significantly less than final germination percentages of cleaned seeds (P<0.001). The control cleaned seeds germinated up to 87%, this was reduced to 65% under moderate water stress (-0.25 MPa, PEG) and 47% in ionic conditions (P<0.001). Germination at low osmotic water potentials of -0.5 MPa (P=0.029) and -1.0 MPa (P=0.004) were significantly higher than seeds germinated in ionic conditions (Fig 11e).

Seed germination of *M. stipoides* was >85% up to -0.5 MPa in both osmotic and ionic conditions. Differences in germination percentages occurred when water stress was moderate-high (i.e. -0.75 MPa), in these conditions germination was >80% except when clean seeds were exposed to NaCl achieving 63% (<0.001). Osmotic conditions were less detrimental to germination in both intact and clean seeds and >80% germination could still be achieved at very low water availability (-1.0 MPa). At -1.0 MPa ionic conditions were unfavourable with only 47% germination achieved in cleaned seeds, keeping the florets intact at this low water potential improved germination up to 70% compared with clean seeds. At water potentials below -1.0 MPa germination was inhibited (Fig 11f).

### 4.5.2 Germination under saline conditions

*A. setacea* germination was significantly reduced at -0.5 and 1.0 MPa compared to the control treatments (P<0.001). All germination treatments reached over 80% germination under non-saline conditions. In moderately saline conditions (-0.5 MPa) germination was reduced to <35% in the control and kinetin treated seeds, while gibberellic acid and salicylic acid appeared to improve the seeds ability to withstand saline conditions. In highly saline (-1.0 MPa) conditions germination was reduced to <10% in seeds treated with water or gibberellic acid. Kinetin and salicylic acid treatments improved germination to 23% and 25% respectively (Fig 12a).

Germination of *A. caespitosa* (Dc1) was >98% across all treatments under non-saline conditions. This reduced significantly in saline conditions (P<0.001). However, under moderately saline conditions germination was still high at >85% across all treatments and 97% in salicylic acid treated seeds, which was significantly higher that the control seeds (P=0.015) and gibberellic acid treated seeds (P=0.017). In highly saline environments germination was around 50% across all treatments (Fig 12b).
Figure 11: Influence of water stress levels on percent seed germination in (a) *Austrodanthonia setacea*, (b) *Austrodanthonia caespitosa* (Dc1), (c) *Bothriochloa macra*, (d) *Chloris truncata*, (e) *Enteropogon acicularis*, (f) *Microlaena stipoides* (Lig 183).

Water stress was imposed by PEG = Polyethylene Glycol, NaCl = Sodium Chloride; Intact = florets intact, Clean = caryopses removed from florets. Germination % indicated by bar columns; white = 0 water potential (ψ), black = -0.25 ψ, white/black dots = -0.5 ψ, grey = -0.75 ψ, white/black stripes = -1.0 ψ, orange = -1.5 ψ.
Chloris truncata appears to be the most saline sensitive species tested. In non-saline conditions germination was >90% across all treatments. In moderately saline conditions low germination of seeds treated with water only was enhanced with gibberellic acid (P=0.017), kinetin (P<0.001) and salicylic acid (P<0.001). Salicylic acid improved germination from 17% to 80%, while gibberellic acid and kinetin improved germination by 34% and 50% respectively. Germination in highly saline conditions was <5% (Fig 12c).

In non-saline conditions gibberellic acid significantly improved germination of *M. stipoides* compared to the control (<0.001), kinetin (P<0.001) and salicylic acid (P<0.001) treated seeds. In moderately saline conditions gibberellic acid, kinetin and salicylic acid improved germination by up to 90% compared to the control (62%). Water treated seeds in highly saline conditions was low at 16%, which was improved significantly by gibberellic acid (70%; P<0.001), kinetin (55%; P=0.002) and salicylic acid (72%; P<0.001) (Fig 12d).

**Figure 12:** Effect of stress tolerance compounds on seed germination under saline conditions in (a) *Austrodanthonia setacea*, (b) *Austrodanthonia caespitosa* (Dc1), (c) *Chloris truncata*, (d) *Microlaena stipoides* (Lig 183).

Water potential $\psi$: 0 = control, -0.5 $\psi$ NaCl, -1.0 $\psi$ NaCl. Germination % indicated by bar columns; white = water only (control), black = gibberellic acid 100ppm, white/black stripes = Kinetin 0.05 mM, grey = Salicylic acid 0.5mM.
4.5.3 Germination under water stress using plant signalling compounds

Germination of *A. setacea*, *A. caespitosa* (Dc1) and *E. acicularis* seeds treated with priming combinations was >80% across all treatments in both water potentials with no major benefit from combining enhancement treatments (Fig 14a, b, d).

*C. truncata* germination at 0 MPa was 68% in control seeds, this was improved to 82% with gibberellic acid and >90% with all other treatments. Priming benefits were observed in -0.5 MPa conditions by combining gibberellic acid and kinetin (95%), salicylic acid (53%), gibberellic acid and salicylic acid (52%) and gibberellic acid, kinetin and salicylic acid (50%). Control seeds germinated to 10% and all other treatments only achieved up to 20% (Fig 14c).

*M. stipoides* germination was 100% in control seeds in both 0 and -0.5 MPa and there was no difference in how seeds responded to priming treatments between 0 MPa and -0.5 MPa (P=0.112). Germination was significantly reduced in the priming combination treatments (except kinetin + salicylic acid) to <80% at 0 MPa (P<0.001), while single priming treatments (i.e. gibberellic acid, kinetin and salicylic acid) germinated to around 90% (Fig 14e). Similarly, germination was significantly reduced in the priming combination treatments (except kinetin + salicylic acid) to <77% at 0 MPa (P<0.001), while single priming treatments (i.e. gibberellic acid, kinetin and salicylic acid) germinated to around 85% (Fig 14e).
Figure 13: Overcoming water stress using seed priming treatment combinations in (a) *Austrodanthonia setacea*, (b) *Austrodanthonia caespitosa* (Dc1), (c) *Chloris truncata*, (d) *Enteropogon acicularis*, (e) *Microlaena stipoides* (Lig 183).

Water stress was imposed using PEG to a water potential of either 0 $\psi$ = control, or -0.5 $\psi$ PEG. Treatments include; white = water only (control), white/horizontal black stripes = gibberellic acid 100 ppm (GA), black = kinetin 0.05 mM (K), white/black dots = salicylic acid 0.5 mM (SA), grey = GA + K, white diagonal black stripes = GA + SA, dark grey = K + SA, white/vertical black stripes = GA + K + SA.
4.6 Seed Coating Technology

4.6.1 Laboratory Trial

Under controlled laboratory conditions film and polymer pellet coating did not provide any extra benefit to seed germination in either intact or cleaned seeds in any of the species trialled (Fig 14). Seed coating decreased germination in *A. caespitosa* Dc1, however no difference was evident in the other *A. caespitosa* line. Similar decreases were observed in *C. truncata* intact seeds (Fig 14c) and *M. stipoides* clean seeds (pellet coat) (Fig 14 e). Under no circumstance was GA detrimental to germination and in some instances (*A. caespitosa* cleaned pellet and *M. stipoides* intact filmcoat) provided a significant increase in germination (Fig 14).

Figure 14: The influence of seed coating on germination under laboratory conditions in Intact (florets intact) or Clean (caryopses removed from florets) of (a) *Austrodanthonia caespitosa* (Bod 12/05), (b) *Austrodanthonia caespitosa* (Dc1), (c) *Chloris truncata*, (d) *Dichanthium sericeum*, (e) *Microlaena stipoides* (Lig 183).

Seedcoats included either a filmcoat = thin film ± germination enhancement treatment, Pellet = thick, shape changing polymer coat ± germination enhancement treatment. Treatments included; black = water only, grey = gibberellic acid 100ppm.
4.6.2 Glasshouse Trial

Germination of surface sown seeds of *A. caespitosa* (WA wild stand collected – Bod 12/05) and *A. caespitosa* (Dc1) was higher than seeds sown at 1 cm depth. Film and polymer pellet coating did not improve germination compared to the control seeds (Fig 15 a, b). The highest germination achieved in *A. caespitosa* (Bod 12/05) when surface sown was 74% for intact control florets and 69% for cleaned control seeds. Germination at 1 cm sowing depth was <5% across all treatments (Fig 15a). *A. caespitosa* (Dc1) germination reached 95% in intact control seeds and 93% in cleaned control seeds that were surface sown. Germination of seed coated either by film and pellet coating had <90% germination when surface. Intact control seeds sown at 1 cm had very poor germination (i.e. <40%), and cleaned control seeds were much worse (8.5%) (Fig 15b). This was not improved by seed coating.

Germination of cleaned seed across all surface sown treatments in *C. truncata* was greater (i.e. >60%) compared to intact seeds in both surface and 0.5 cm sown seeds. Germination of seeds remaining in the floret was very poor (<10%) across all treatments (Fig 15c). Seed coating did not appear to have a significant negative impact on *C. truncata* germination.

*D. sericeum* germination was enhanced from 30% in the intact control surface sown seeds to 34% when seeds were coated with a film containing gibberellic acid (P <0.05). Cleaned seeds also benefited from gibberellic acid film coating and germination improved from 14% in the control to 18% with coating. Seeds sown at 1cm had very poor germination (<7%) across all treatments (Fig 15d).

Surface sowing seeds of *M. stipoides* was more successful than sowing seeds at 1cm depth under glasshouse conditions. Germination of intact seeds was >70% across all treatments, except clean, film coated seeds sown at 1cm where only 3.5% was achieved. The highest germination of intact florets was 87% in the control sown at 1cm. Cleaned surface sown control seeds were improved from 85% to 91% when film coated (Fig 15e).
Figure 15: The influence of seed coating on germination under glasshouse conditions in Intact (florets intact) or Clean (caryopses removed from florets) of (a) Austrodanthonia caespitosa (Bod 12/05), (b) Austrodanthonia caespitosa (Dc1), (c) Chloris truncata, (d) Dichanthium sericeum, (e) Microlaena stipoides (Lig 183).

Seedcoats included either a filmcoat = thin film ± germination enhancement treatment, Pellet = thick, shape changing polymer coat ± germination enhancement treatment. Treatments included; black = water only, grey = gibberellic acid 100ppm.

4.6.3 Field Trial

Seed coating technology did not improve germination of A. caespitosa (Bod 12/05), C. truncata or D. sericeum in the field trial (Fig 16 a, c, d). Germination across all treatments was either extremely poor or absent. A. caespitosa (Dc1) germinated better when sown at 1 cm compared to surface sowing.
Germination was improved in intact control seeds from 22% to 48% by burying seed. Clean seeds that were surface sown had no germination; this was improved by 37% when seeds were buried. The best treatment was film coating with either water (52%) or gibberellic acid (60%) sown at 1 cm (Fig 16 b).

Surface sown seeds of *M. stipoides* had practically zero germination (the highest being intact control seeds with 6%) across all treatments. This was dramatically increased by sowing seeds at 1 cm depth. Intact control seed germination improved by 67% and clean control seeds by 87%. The best treatments were film coated intact seeds (81%) and clean control seeds (88%) (Fig 16 e).

![Figure 16](image)

Figure 16: The influence of seed coating on germination under field conditions in Intact (florets intact) or Clean (caryopses removed from florets) of (a) *Austrodanthonia caespitosa* (Bod 12/05), (b) *Austrodanthonia caespitosa* (Dc1), (c) *Chloris truncata*, (d) *Dichanthium sericeum*, (e) *Microlaena stipoides* (Lig 183).

Seedcoats included either a filmcoat = thin film ± germination enhancement treatment, Pellet = thick, shape changing polymer coat ± germination enhancement treatment. Treatments included; black = water only, grey = gibberellic acid 100ppm.
5. Discussion

5.1 Germination Enhancement

Improvements in germination by seed treatments in this study were shown to be species, accession and indeed collection location specific. Despite this, there appears to be several seed treatments that provide a general benefit to several species. Under controlled conditions the removal the caryopses from florets increases germination. This is in agreement with previous research that indicates the protective structures of native grass seeds can contribute to seed dormancy in some species (Tothill 1977; Lodge & Whalley 1981; Silcock et al. 1990; Adkins et al. 2002; Lodge 2004). Floret removal in the present study involved careful seed dissection by hand or by rubbing out with soft rubber mats. If naked caryopses are to be utilised in broad acre restoration, techniques need to be developed to scale up seed cleaning. This will be of particular importance for seeds like *M. stipoides* that require extreme caution in floret removal but which will return significantly increased germination (Fig 5c).

Primming technologies satisfactorily overcame seed dormancy under controlled environment conditions. For example seeds primed with gibberellic acid promoted the germination of 22 accessions (10 species) out of the 53 accessions tested (Table 2). Gibberellic acid is a growth hormone that promotes the elongation of cells and can stimulate germination or break dormany in seeds that normally require after-ripening, or are physiologically dormant. Smoke water also promoted germination in 14 accessions (7 species) of native grasses tested. Smoke has been shown to have a similar mode of action to GA in stimulating germination of native species. Previous research indicates that some native grass species respond well to smoke treatments (Read & Bellairs 1999; Clarke & French 2004) and can overcome physiological dormancy (low growth potential of the embryo), a characteristic of the Poaceae family (Baskin & Baskin 1998).

Two of the C4 species (*Chloris truncata* and *Themeda triandra*) responded to a heat shock treatment, which is known to overcome after ripening requirements of seeds. Research has shown that continuous summer temperatures (32°C/11°C day/night temperature) over three weeks is optimal for germination of C4 summer growing species (Hagon & Groves 1977). Further research is required to determine whether exposure to high temperatures (i.e. 80 -100°C) for short periods can also overcome after-ripening in other C4 native grass species.

The ability to treat large amounts of seed with this priming technology is an important next step in optimising seed quality for use in broad acre restoration. The principles behind the priming process are simple. However, optimising the process for industry (cost, time, environmental conditions) still needs to be done if the process is to be practical.

5.2 Field Trials

5.2.1 C3 Species

In this study germination was improved under laboratory, glasshouse and field experiments compared to the industry standard of sowing seeds intact on the soil surface. The field study focussed *A. caespitosa* and *M. stipoides* due to insufficient seeds of other species. Under laboratory conditions treatments improved final germination percentage above 80% in both of the species and in all accessions tested. Germination under glasshouse conditions was slightly less than under laboratory conditions however; germination was increased from 53% to 75% in *A. caespitosa*, 58% to 64% in *M. stipoides* (Griffin) and from 61% to 73% in *M. stipoides* (Ligule 183) with either seed cleaning or GA being the most consistent treatments. Improvements in final germination percentage under field conditions of up to 53% and 55% were achieved for *A. caespitosa* and *M. stipoides* respectively. The best bet treatment for *A. caespitosa* was determined to be GA priming intact seeds buried at 1cm and for *M. stipoides* the best bet treatment was to have cleaned seed buried at 1cm (Fig 5).
From the results it is clear that the field surface sown seeds do not germinate as successfully as seeds that are buried at 1cm depth. Intact florets germinate better in the field than naked caryopses when surface sown, possibly due to the floret appendages retaining moisture resulting in more favourable seed water relations for germination. Likewise, soil water relations in glasshouse studies were likely to be more favourable for germination compared to field studies where soil drying from wind and sun will be more prevalent. This may partly explain the higher germination of surface sown naked caryopses in the glasshouse. Future research will need to focus on soil conditions that can optimise seed water relations or on improving seed germination performance under water stress conditions.

5.2.2 C4 Species

Germination was improved under laboratory, glasshouse and field experiments (Table 2). Under laboratory conditions, treatments improved final germination percentage above 60% in *C. truncata* (cleaned seed plus GA priming) and above 90% in *D. sericeum* (cleaned seed plus GA priming) (Table 2). Germination under glasshouse conditions was slightly less than under laboratory conditions, however germination was increased from 41% to 86% in *C. truncata* (GA or KAR1 primed cleaned seed sown on the surface). Under field conditions, final germination percentages of up to 29% were achieved for *C. truncata*. The best bet treatment was priming heat treated seeds with KAR1 prior to burial compared to the control of 12%. GA was also another treatment that showed potential (up to 25% germination). For *D. sericeum* field emergence up to 67% was observed for GA treated intact seeds sown at 1cm. This resulted in a germination rate almost 2 fold greater than any other treatment.

From the results it is clear that under glasshouse and field conditions *C. truncata* germination and emergence can benefit from seed cleaning, surface sowing and priming with gibberellic acid or karrikinolide. Unlike other trialled species, *Chloris truncata* floret appendages do not appear to protect the seed from desiccation under field conditions, possibly due to a comparatively small seed size requiring less water to enable seed imbibition and germination allowing for immediate cell elongation and growth. Also, with the benefit of germination enhancers such as gibberellic acid and karrikinolide the speed of germination is further increased.

Interestingly, *D. sericeum* performed more similar in the glasshouse and the field compared to other species. Intact florets treated with gibberellic acid were by far the most successful combination for improved germination and emergence. In the glasshouse, sowing depth did not appear to influence germination success but under field conditions, burying seeds was preferable. Under field conditions, soil moisture relations may be more favourable within the soil with less risk of drying from wind and sun, which may explain why glasshouse seeds germinated successfully whether surface or 1cm sown as the drying effects are minimised due to the protected conditions. Also, removing seeds from the florets has the potential to damage the caryopses minimising the ability for seeds to germinate successfully.

The interaction between soil traits (moisture retention and drying susceptibility) and seed treatments is likely to be an important factor in determining the suitability of these treatments for broad scale usage. Like the species used in the C3 trial, future research will need to focus on soil conditions under water stress but also optimising seed-soil water relations (i.e. interactions of soil type, sowing depth and climate) and how this may influence the success and speed of germination and emergence.

5.3 After-ripening and storage

All four species possessed at least partial dormancy before being placed in after-ripening conditions. Under these conditions *T. triandra* and *D. sericeum* did not show improvements in germination over the 3 month period. *A. setacea* was reduced after 1 month and *C. truncata* germination was reduced after 2 months in these conditions (45°C, 50% RH). For some species (*A. setacea, C. truncata, D. sericeum* and *T. triandra*) the after-ripening conditions utilised were limited to one temperature and RH% that were thought to induce rapid aging of the seeds. It is likely that other after-ripening conditions may be more suitable (i.e. less detrimental over the short term), and provided seed is not a
limiting factor, curves should be developed for a range of temperatures and RH’s to identify optimal after ripening conditions. The fact that there are species differences under these extreme storage conditions highlights the importance of treating each species separately.

For the two species \textit{M. stipoides} and \textit{A. caespitosa} other RH’s and storage temperatures were examined. Seeds of \textit{A. caespitosa} (Dc1) stored at temperatures of 5°C and -18°C and 50% RH appeared to benefit from 6 months storage, while germinability was reduced in seeds stored at 23°C. The most significant finding was that cleaned fresh \textit{A. caespitosa} seeds maintained a significantly higher germination potential than intact seeds up to 6 months after storage at -18°C, 5°C and 23°C at both 20% and 50% RH. Cleaned seed viability, however, declined at all storage temperatures after 6 months at 20% RH. \textit{M. stipoides} viability remained high after 12 months at low temperatures with some decline in intact seeds and a significant loss in viability in cleaned seeds stored at 23°C, 50% RH.

After-ripening has shown to be important in species that were thought to possess deep dormancy. For example, Wells and Dixon (2003) found that \textit{Triodia} species, once thought to be deeply dormant, were able to germinate when seeds were stored for 12 months in ambient conditions. It appears that seed dormancy and after-ripening requirements are species specific, requiring particular conditions before dormancy is broken. Groves \textit{et al} (1982) found that populations of \textit{Themeda australis} collected from across Australia had different levels of dormancy between populations, attributed to maternal environment effects.

Future research should be directed towards determining whether genetic, or maternal environmental conditions of native perennial grasses are fundamental to successful germination and establishment. Also, accelerated after-ripening experiments using various temperatures and relative humidity could be implemented to determine variations between species and dormancy length. Previous research also suggests that alternating storage temperatures may facilitate germination in some Poaceae species, for example, Hagon (1976) found that \textit{Themeda australis} and \textit{Austrostipa} sp. germination could be increased substantially after seed storage in alternating warm or cold temperatures. Reducing moisture content is also an important factor associated with the optimal storage of seeds and research suggests that it is the most important determinant of longevity in storage (Wilson 1995, Merritt 2003).

5.4 Stress Tolerance

This study highlighted large differences between species in their ability to germinate under drought/saline conditions. \textit{Microlaena stipoides} was shown to be extremely resilient to osmotic stress (induced by PEG) and salt stress (NaCl), whilst others like \textit{E. acicularis} was determined to be water stress sensitive. Differences within genera were also observed with \textit{A. setacea} being significantly more susceptible to salt than \textit{A. caespitosa}. With the need to find salt and drought tolerant species for restoration of degraded agricultural lands some native grasses may offer a suitable alternative to other more studied plant species. A drought/salt tolerance screening process (particularly focussing on \textit{M. stipoides}) should be considered to identify tolerant ecotypes, both at the germination phase and at the early seedling and adult phase. \textit{Given M. stipoides} appears to have a robust tolerance to drought/salt at the early germination phase, determining the mechanisms of tolerance may be beneficial to identify key tolerance traits.

The tolerance of native grasses was also shown to be improved through priming seeds with stress tolerance agents including kinetin and salicylic acid. These compounds improved germination in three out of the four native grass accessions (two out of three species) tested. It appears that these chemicals provide a means to improve germination under drought conditions at minimal risk. As the mode of action of these chemicals in conferring stress tolerance is not known at present, more work is required to test the robustness of these treatments under field conditions before a final recommendation can be made.
5.5 Seed coating

In this study the ability of seed coats to deliver germination signals under controlled environment and field conditions was examined. Overall seed coatings were shown to produce germination results similar to uncoated seeds, indicating that germination stimulants supplied by artificial coats provided no added benefit to field establishment. That said, the role of seed coating in providing benefits to field establishment of native grasses should not be dismissed. The ability of seed coats to aid in seed handling may indeed result in more efficient plant establishment at much lower seeding rates/costs. Further research under a range of field conditions (soil types, environments) interacting with seed to site delivery mechanisms will demonstrate the role of seed coats in broad acre agriculture. If seed coats can not effectively transfer the necessary signals to the seed to promote germination, but are required for adequate seed handling a combination of seed priming and coating may be necessary, provided the system is simple and cost effective.
6. Implications

This research has direct implications for the native grass industry, particularly for suppliers and collectors of native seed. Relatively simple and cheap seed treatments produced significant improvements in native grass germination and were useful across many species/accessions tested. The approach used in this report can be readily adapted to industry to improve the quality of the end product. This quality increase will hopefully facilitate broad scale adoption of native grasses in Australia.

Best bet treatments for several key native grass species have been identified including:

- *A. caespitosa* - GA priming intact seeds buried at 1cm
- *M. stipoides* - cleaned seed buried at 1cm
- *C. truncata* - priming seeds with KAR₁ or GA prior to burial
- *D. sericeum* - GA treated intact seeds sown at 1cm.

If these findings are to be adopted for broad scale industry use, this technology needs to be scaled up ensuring end users are able to cost effectively treat their seed.
7. References


Rehabilitation of degraded lands through the use of native grasses is likely to enhance productivity in poor performing agricultural and mining rehabilitation areas, thus improving industry sustainability by increasing biodiversity and restoring ecosystem function. At present, variable and unsuccessful native perennial pasture establishment is the result of low availability of viable seed, asynchronous and unreliable seed germination, poor seedling emergence and low seedling vigour.

This project targets the development of a national program to deliver seed-based solutions to overcome seed germination and establishment barriers for a profitable and productive broad-acre native perennial grass pasture and restoration industry.

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