Department of Environment and Agriculture

Conservation of Arid Plants through Improved Understanding of Seed Biology as a Means of Enhancing the Functionality of Botanic Gardens

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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.

Human Ethics

The proposed research study received human research ethics approval from the Curtin University Human Research Ethics Office, Approval Number HRE2017-0735.

Alaa Shallal Nayyef

7 July 2018

Statement of Contribution by others

I, Alaa Shallal Nayyef, carried out all work described in this thesis including the literature review, research design planning, methodology, plant and seed investigation, data curation and analysis, botanical garden survey and writing with the advice and support of my supervisors: Dr. Deborah Pritchard, Dr. David Merritt and Dr. Shane Turner.

Alaa Shallal Nayyef (PhD candidate)

Deborah Pritchard (Primary supervisor)

Abstract

Around the world many plant species are threatened by extinction and habitat loss particularly in dry arid lands due to the extreme environmental conditions and human disturbances leading to desertification. The Pilbara region in the north of Western Australia is one of the oldest biodiverse landscapes in the world with a semi-arid tropical climate. Mining, which is an important industry in this region, has been one of the main activities that has contributed to the loss of many plants species and requires active restoration for the disturbed areas following mine-site closure. Seeds are considered an important element in land restoration, however many species have poor germination and seedling emergence when used in restoration through either possessing seed dormancy or poorly resolved germination requirements developed as an adaptation in response to low irregular rainfall and high temperatures

This study identifies the seed biology and germination requirements of seven species from three different native understory families (Cyperaceae, Goodeniaceae, Poaceae) that contribute to mine-site restoration in the Pilbara region. The first step of the study was to identify seeds and embryo characteristics and test seed coat permeability to water. A germination test using different incubation temperatures and germination stimulants was then conducted to establish which species were germinable and those that possessed seed dormancy. The experiments showed that seeds of *Eragrostis eriopoda*, *Fimbristylis dichotoma* and *Goodenia stobbsiana* were identified as having physiological dormancy while seeds of *Cymbopogon obtectus*, *Eriachne mucronata*, *Goodenia armitiana and Goodenia cusackiana* were found to be largely non-dormant germinating over a range of conditions. Additionally, seeds of *Cymbopogon obtectus* and *Eriachne mucronata* were also tested for germination with and without florets where it was found that germination of *Eriachne mucronata* decreases with the presence of florets but the seeds of *Cymbopogon obtectus* were unaffected by the surrounding florets germinating to >90% within 7 days.

The second step was to overcome seed dormancy through several experiments that investigated afterripening and wet/dry cycling under different temperatures and durations and by using acid scarification for different durations and concentrations and mechanical scarification under various grit sizes and time combinations. Compared to other dormancy alleviating treatments investigated during this study both forms of scarification proved to be largely ineffective in overcoming seed dormancy though acid scarification was

relatively more successful than mechanical scarification. Afterripening at 50°C and 50% RH for 26 weeks improved germination in *Eragrostis eriopoda* seeds when incubated at 25/45°C which proved to be the most effective treatment for overcoming dormancy in this species. In comparison, wet/dry cycling at 30°C and 50% RH for 18 months improved germination of *Fimbristylis dichotoma* seeds when incubated at 40°C. Both species showed a strong positive response to high temperature (~40°C) incubation suggesting that these two species require hot germination conditions compared to the other species assessed in this study. Afterripening at 30°C and 50% RH for 8 months was found to increase germination of *Goodenia stobbsiana* seeds when incubated at 25°C in the presence of KAR_1.

Enhancing the germination of non-dormant seeds was another target for this study with seed priming investigated as one possible way to improve the germination parameters of *Cymbopogon obtectus* and *Eriachne mucronata* seeds. For both species seeds were primed in aerated water for 3 hours, 6 hours, and 9 hours at room temperature (~23°C) and then tested for germination at 30°C under different water potentials (0, -0.25, -0.5, and -1.0 MPa) to simulate nil to severe moisture stress. In response to these germination conditions it was observed that certain priming treatments proved to be useful in enhancing overall germination of *Cymbopogon obtectus*, the rate of germination as well as improving the tolerance of germinating seeds to high moisture stress of *Cymbopogon obtectus* and *Eriachne mucronata* seeds.

As a final contribution to improve arid land restoration and plant conservation a botanic gardens survey was undertaken and analysed to better understand how conservation and land restoration programs are implemented and managed by different botanic gardens across the world. This was done through a survey directly sent to many botanic gardens from different regions that contained 30 multiple choice and short worded questions. Results from the survey defined and outlined many structures, trends, strengths and weaknesses of botanic gardens. The findings have improved our knowledge of botanic gardens in arid regions to assist in future planning to support and promote their development in a number of key conservation, restoration, education and public engagement functions.

Overall, this study provides information to assist restoration programs in arid lands particularly in the Pilbara region, with findings applicable for other arid lands that experience high temperatures and low irregular rainfall. The findings can be used to improve the functionality of botanic gardens from different environments; particularly

those from semi-arid regions through achieving a better understanding of strategies used by botanic gardens in conservation research and provide a frame work for the future planning and design to better serve their local communities.

Table of Contents

List of Figures

List of Tables

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Chapter 1 General introduction and literature review

1.1 Introduction

Globally there has been a decline in natural habitats in arid regions (Sinclair et al., 1995; Vitousek et al., 1996; Mahmoud and Gan 2018). Desertification, which is the process by which relatively fertile lands become denuded of vegetation and associated biotic communities, is common in arid zones and is a serious problem affecting the survival of many plants endemic to these regions (Gutterman, 2012). A number of arid regions worldwide have little provision for the protection of declining and threatened plant communities. There is a need for countries affected by desertification in arid and semi-arid zones to develop strategies to conserve and protect their endemic plant communities. Revegetation of arid and semi-arid land is difficult because it is widely influenced by extreme climatic factors, disturbance, limited revegetation technology and importantly a lack of understanding of the biology and ecology of native plant species (Call and Roundy, 1991).

Botanic gardens are found world-wide fulfilling important functions such as documenting the collection and taxonomy of living plants for the purposes of scientific research, conservation, display and education. A key increasing feature of a botanic garden is its role in restoration through the collection, curation and utilization of native seeds for conservation and land rehabilitation purposes. Most restoration projects consider top soil as its principle resource for supplying seeds, plant propagules, nutrients and microbes so the respreading of stripped topsoil is one way to commence restoration works (Rokich et al., 2000; Koch, 2007a; Bainbridge, 2007; Golos et al., 2016). The use of topsoil in revegetation projects can also be problematic as in many cases it is often no longer viable as it has been stockpiled for too long (>12 months), does not contain the range of species that are needed to fully restore the vegetation community or there is insufficient quantity available to meet requirements so other approaches such as direct seeding and the planting of greenstock may be necessary (Koch, 2007b; Golos et al., 2016; Erickson et al., 2017). Nevertheless for directly sown seeds, the germination of plants in arid zones is commonly low due to suboptimal soil conditions (i.e. lack of soil moisture), predation or inherent seed dormancy; a major barrier to land restoration with over 70 % arid zone species having seeds with some form of dormancy (Erickson, 2015; Erickson et al., 2017). This poor seed

performance is further exacerbated by a lack of taxonomic understanding and poor survey data from many arid environments (Keighery, 1996). The principle aim of restoration is re-establish species and ecosystem. Climate change and human disturbance such as mining both impact on restoration specifically on plant reproduction through affecting seeds availability and viability (Broadhurst, et al., 2016). Seed dormancy under these conditions limit the opportunity to develop proper strategies to secure sufficient amount of seeds to achieve successful restoration.

1.2 Arid and semi-arid land

1.2.1 Regions and climate

Arid areas receive an annual mean rainfall less than 500 mm while semi-arid area receive 200-500 mm with factors such as temperature, humidity and light also important in regulating the type of floristic communities that grow in these types of environments (Williams, 1999; Grainger, 2013). Aridity is an ecological condition where water income is less than potential water expenditure such as runoff and evapotranspiration (Dave et al., 2019). Arid lands may differ in their climate as some can be hot, such as the Sahara Desert in the North-East of Africa and The Arabian Desert in Western Asia; moderate, such as Atacama Desert in South America, or; cold, as The Great Basin Desert in The United States. However, they all share one trait which is that they are dry, to very dry for most of the time (Pellant, 2004; Edgell, 2006; Bainbridge, 2012; Zhang, 2014, Oerter, 2016). The major issue in arid lands is desertification, a land deterioration problem which affects nearly all arid regions but to differing degrees (Dregne, 1986). Desertification is defined by two main characteristics; degradation of soil and loss of vegetative cover (Grainger, 2013). There are many possible reasons leading to the degradation of both plants and soil and these may be indirect factors such as population pressure, socioeconomic and policy and/or direct factors such as land use practices and climate-related processes (Dregne, 2002; Millennium Ecosystem Assessment, 2005). More than 40% of arid lands are classified as extremely degraded that in all likelihood can only be recovered with direct human intervention (Daily, 1995). Land degradation is not a new phenomenon and happens gradually, over the last 300 years the average loss of soil due to degradation was 200 million tons per year (Rubio, 2009).

Hence, given the widespread desertification of arid regions and the fact that they cover more than one-third of the earth land surface (Grainger, 2013) there is a need to have

conservation plans and strategies in place to both protect and expand (where possible) native plant species under both *in situ* and *ex situ* conditions. Despite the fact that the importance of arid land restoration is well recognized around the world the rate of successful restoration is still low (James et al., 2013).

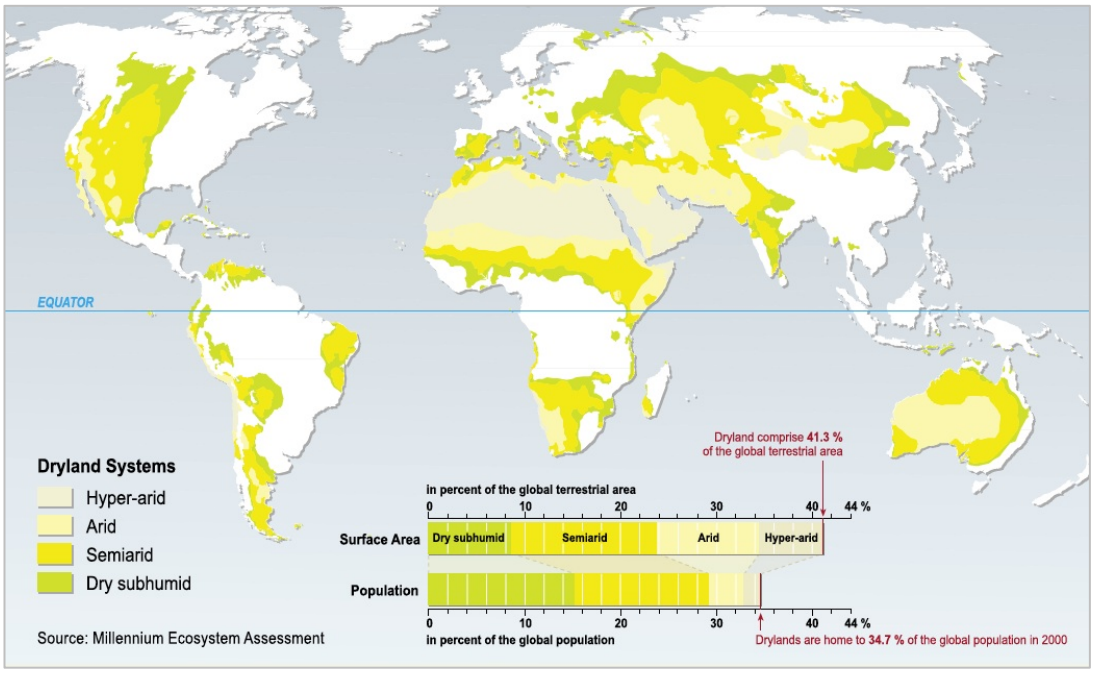

Figure 1.1. Global map of major dryland areas.
(Millennium Ecosystem Assessment, 2005)

1.2.2 Features of plants in arid regions

1.2.2.1 Key plant groups of arid regions

Three groups of vegetation can be distinguished growing in arid lands. The major group are non-succulent perennials which contain grasses, woody herbs, shrubs, and trees that tolerate the harsh environment and arid conditions. The other two groups are ephemeral annuals which are transitory, only appearing after rains for a short period of time (i.e. annual grasses and daisies), and succulent perennials that store water and have low transpiration (Salem, 1989). Shrubs are the most dominant plant form in arid areas while some tree species show a decrease in height and may be classified as shrubs (Baskin and Baskin, 2014). Grasses are highly valuable in arid lands as they have the ability to capture water through slowing down its movement and assisting the entry of water into the soil by the root and soil organisms associated with grass communities (Anderson and Hodgkinson, 1997). Grasses also protect the soil and help to stabilise mobile dunes and

loose sand from damaging winds and are important fodder and habitat for many native animals (Yang et al., 2006; Letnic and Dickman 2010).

1.2.2.2 Environmental factors responsible for dormancy break and germination

Seed dormancy is a trait earned through evolution in order to survive adverse conditions such as heat, cold and drought (Bradford and Nonogaki, 2007). Seed dormancy also prevents premature germination of the embryo before dispersal and provides adaptive advantage for spreading by wind, water or other dispersal means (Simpson, 1990). Seeds in the soil seed-bank constantly alter and adjust their dormancy status by sensing a range of different environmental signals that may indicate the most suitable conditions to germinate (Figure 1.2). Species respond to environmental signals in different ways depending on the native habitat and climate (Finch-Savage and Footitt, 2015). However, not all seed characteristics have a role in releasing dormancy (Benech-Arnold et al., 2000). The conditions that break dormancy and stimulate germination are those that indicate suitability for seedling establishment. From a seed germination perspective it is important to understand the environment into which seeds are dispersed, as mimicking the conditions they receive before, during and perhaps after the growing season can alleviate dormancy. For example, storing air-dried seeds at room temperature (after ripening) can break dormancy of some desert seeds which mimics physiological dormancy loss that occurs in the soil seed bank during a hot dry summer (Baskin and Baskin, 1998).

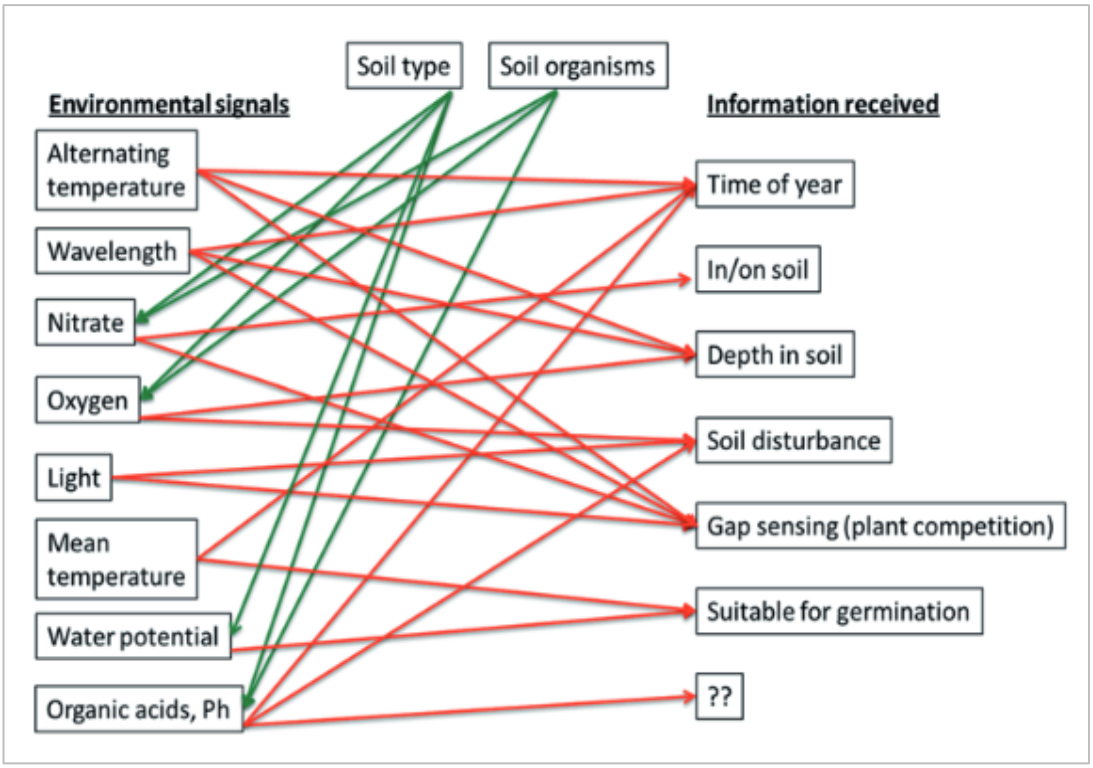

Figure 1.2. Different environmental signals and the indications they provide to seeds *(Finch-Savage and Footitt, 2015)*

1.3 Seed germination and dormancy

1.3.1 Seed germination

Seeds are a small individual system that allows plants to disperse and regenerate and assure their ongoing survival. Seeds consist of an embryo, which in most cases is formed by sexual reproduction, and contain an embryonic axis (i.e. root and shoot apices) and one or more cotyledons surrounded by a seed coat (testa) with endosperm and perisperm commonly present as well (Bewley et al., 2013). Plant species produce seeds that differ in their external morphology such as size and shape and coat surface but seeds may also differ in the internal morphology such as the position, size and shape of the embryo. An early seed classification of inner seed morphology was developed by Martin (1946) and recognised 10 types based on embryo and endosperm characteristics and two other type based on seed size (dwarf and micro). More recently it has been modified by Baskin and Baskin (2007) (Figure 1.3).

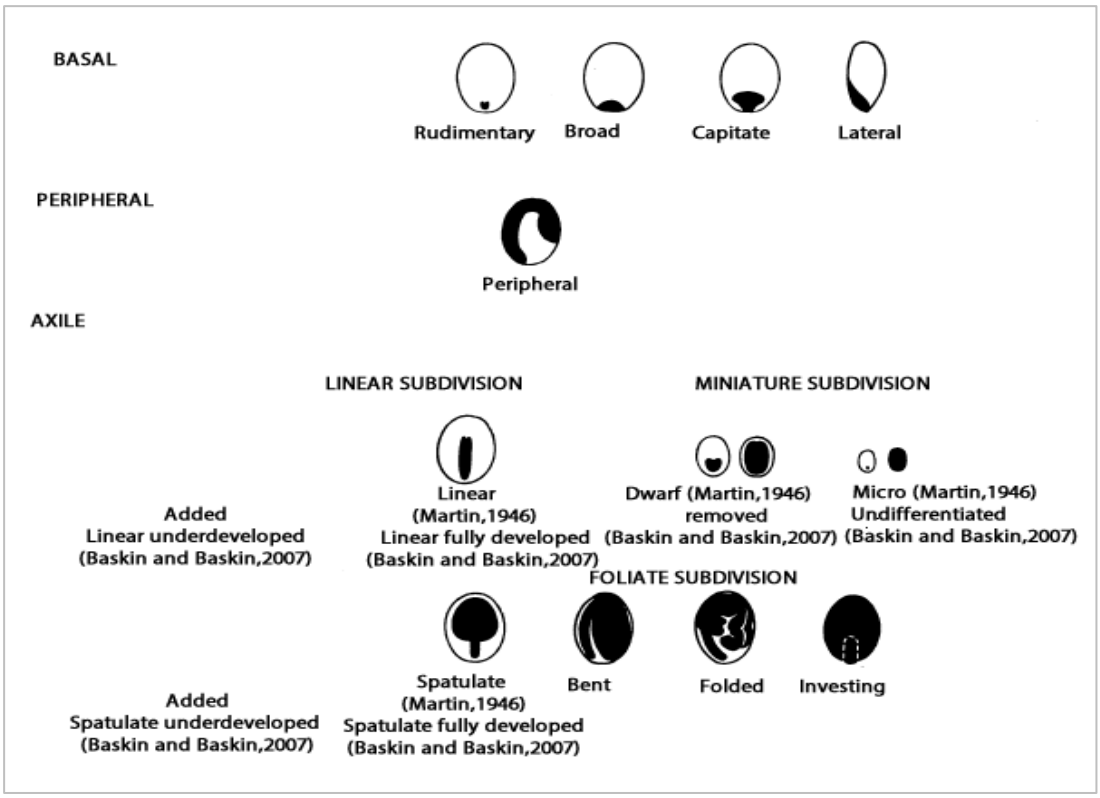

Figure 1.3. The comparative internal morphology of seeds (Martin, 1946) modified by Baskin and Baskin (2007).

Baskin and Baskin (2007) modified Martin's embryo classification system through removing dwarf embryo type and replacing the micro type with "undifferentiated" and by adding an under-developed embryo type to fully developed linear embryos and adding spatulate under-developed embryos to the fully developed spatulate embryos. Morphological diversity is important in classifying seeds and to obtain a better understanding of their dormancy alleviation and germination requirements.

Germination is considered a critical stage in the life cycle of all seed-bearing plants (Bradford and Nonogaki, 2007). Germination begins with the passive uptake of water by the quiescent dry seed and terminates with the elongation of the embryonic axis and is considered to be a triphasic process (Bewley and Black, 1994) (Figure 1.4). The First phase starts with the rapid uptake of water and solute leakage caused by structure disturbance within the imbibing seed, followed by a more stable phase through less water uptake and metabolic activity, with the third and final phase defined by radicle elongation completing the germination process (Bewley, 1997). However, the last phase cannot be reached by most dormant seeds even though dormant seeds may pass through the first and second phases (an exception being seeds that have physical dormancy).

Seeds of different species have individual environment requirements for germination (Baskin and Baskin, 1998). Moisture is an essential factor and considered as the trigger to start germination, while a suitable temperature affects the amount of water imbibed and key metabolic processes (such as the translocation of nutrients and hormones, cell division and elongation and oxidation of starch, fats and proteins) within the seed (Pessarakli, 2001). Seeds in arid lands have different types of adaptations that affect and regulate the timing of germination and seedling recruitment (Erickson et al., 2016). Most plants species from the desert zone produce seeds that are dormant at maturity, with physical and physiological seed dormancy the most common in desert plant species including those found in the Pilbara (northern Western Australia) (Baskin and Baskin, 1998; Facelli et al., 2005; Erickson et al., 2017). Around 70% of Australian plant species maintain some type of seed dormancy and require different conditions to germinate (Merritt et al., 2007; Turner and Merritt, 2009). Physiological dormancy is the most prevalent class of dormancy in the Australian flora and appears to be of similar importance worldwide as well (Baskin and Baskin, 1998; Merritt et al., 2007; Erickson et al., 2017).

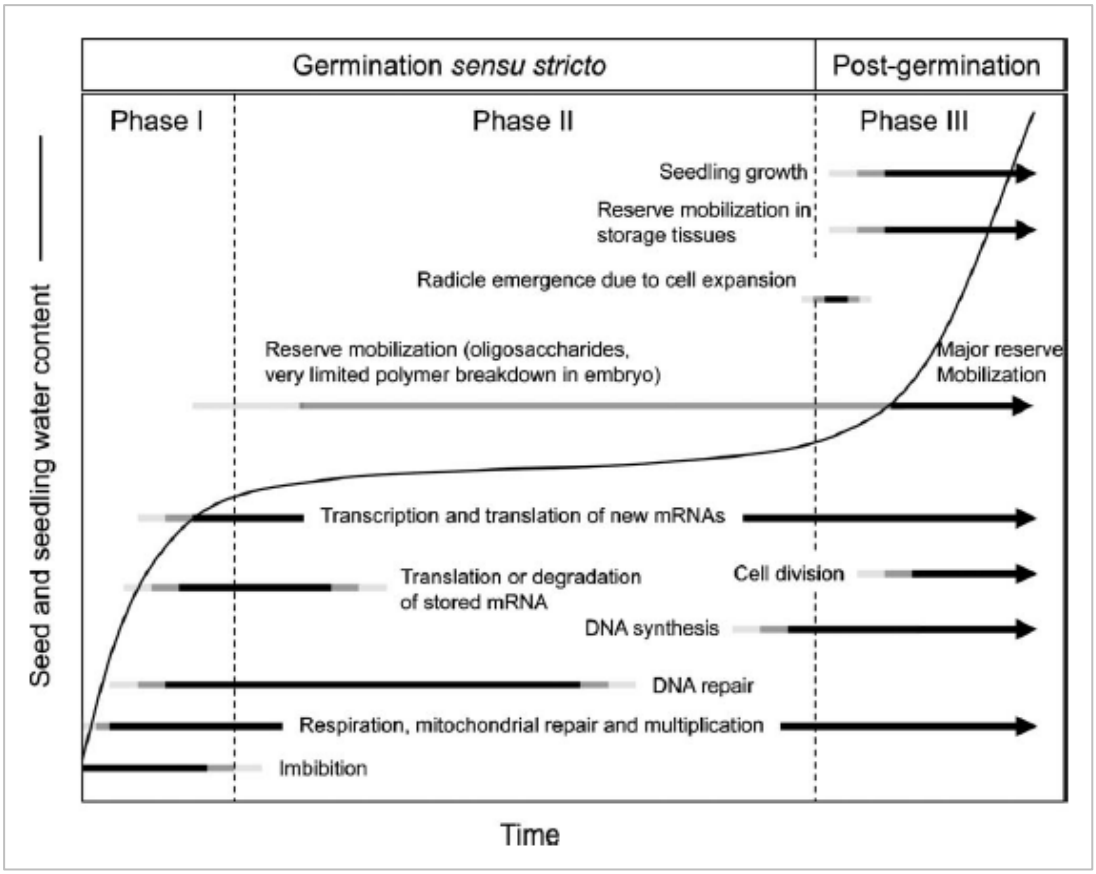

Figure 1.4. Conceptual diagram of the seed germination process
The three phases starting by rapid uptake of water followed by the second phase through less water uptake and the beginning of metabolic activity, then the third and final phase defined by cell division and radicle elongation which completes the germination phase (Nonogaki et al., 2010).

1.3.2 Seed dormancy

Many definitions have been proposed to describe seed dormancy. Seed dormancy is a complicated trait since it is difficult to define the site of action (embryo, endosperm or surrounding tissues), the time it is imposed (during seed maturation or during imbibition of seeds) and the extensive effect of the environment (Bentsink and Koornneef, 2008). Some studies determine dormancy as a failure of viable seeds to complete germination under conditions perceived to be favourable for germination (Bewley, 1997; Bentsink and Koorneef, 2008; Simpson, 2007; Bradbeer, 2013). Vleeshouwers et al. (1995) and Finch-Savage and Leubner-Metzger (2006) define seed dormancy as 'The characteristic that define the conditions required for germination' which is helpful for the selection of suitable conditions for germination and consequently establish the next generation of plants.

The views of various authors however, contrast over the conditions that affect dormancy and germination (Thompson and Ooi, 2010). The interaction between the required conditions that terminate dormancy and those that stimulate germination is controversial and it can be hard to define where dormancy ends and germination begins. Vleeshouwers et al. (1995) state that only temperature can affect dormancy release while light and nitrate promote/stimulate germination, whereas Finch-Savage and Leubner-Metzger (2006) consider light to overcome seed dormancy in addition to temperature. Similarly, Baskin and Baskin (2004) agree and define a dormant seed as "one that does not have the capacity to germinate in a specified period of time under any combination of normal physical environmental factors (temperature, light/dark etc.) that are otherwise favourable for germination". Merritt et al. (2007) distinguish between the factors responsible for the alleviation of dormancy such as time, temperature and moisture and the ones that stimulate germination such as light, nitrate and smoke which can only influence germination once dormancy has been alleviated. Similarly, Thomson and Ooi (2010) consider the requirements for dormancy alleviation fundamentally different from germination stimulation. Amen (1966), Vleeshouwers et al. (1995) and Fenner and Thompson (2005) claim that the function of dormancy is to allow a full life cycle for plants (i.e. plants reach reproductive maturity) where conditions may be suitable for germination but affect survival and growth in the longer term so are unlikely to be reproductively successful. Seed dormancy allows species to persist in the soil seed bank until dormancy is broken and conditions are suitable for germination, emergence, plant growth and establishment thus avoiding suboptimal growing conditions (Finch-Savage and Leubner-Metzger, 2006).

Germination happens as a response to environmental requirements while dormancy break occurs when changes happen within the seeds and are mainly responsible for determining germination requirements (Thompson and Ooi, 2010). Plant hormones are important in regulating dormancy and germination. Gibberellins (GA) have a key role in releasing dormancy and promoting germination (Kucera, 2005). It was suggested that GA opposes the effect of Abscisic acid (ABA), increase the growth potential of embryo, release and trigger the weakening of seed covering layer (Bewley, 1997a, b; Koornneef et al., 2002; Leubner-Metzger, 2003b). Smoke is well known to promote germination in different habitats but mainly in species with physiological dormancy (Erikson et.al, 2016b). Karrikins (KAR1) 3-methyl-2H-furo[2,3-c]pyran-2-one is the primary seed germination stimulants in smoke and it can trigger the germination of different species from fire prone regions, such as the Pilbara. Seeds from different species have variable temperature requirements for germination (Baskin and Baskin 2014), in arid and semi-arid land the time of rainfall is

coincide with optimal temperature (Bell 1999). In fire prone regions, plants have different traits to survive and germinate (Commander et al., 2017), Fire cues, like heat and smoke derived chemicals KAR1 and glyceronitrile (Flematti et al., 2004, 2011) can act singly or in combination as a triggers in fireprone ecosystems (Nelson et al., 2012) to encourage germination (Tieu et al., 2001). It was found that some species also may be stimulated by KAR1 even if they are not been known to be responsive to smoke or fire, such primary dormant Arabidopsis seeds. Natural diversity and the extent of seed dormancy however, are the factors that impact the degree of KAR1 stimulation (Nelson, Riseborough et al., 2009).

1.3.3 Dormancy classification

There are different types of dormancy in seeds with several classification systems developed over many years to facilitate a better understanding of seed dormancy (Lang et al., 1987; Bewley and Black, 1994; Nikolaeva, 2001; Baskin and Baskin, 2004a). The most comprehensive classification system is the one proposed by Nikolaeva (2004) which is based on the causes of dormancy and the conditions required to break it. Nikolaeva´s classification system contains three dormancy types: exogenous, endogenous and a combination of the two. Exogenous dormancy is related to outer seed coverings (e.g. the seed coat) and falls into three categories: physical, chemical and mechanical. Endogenous dormancy is a property of the embryo and includes morphological and physiological dormancy (and a combination of these), while the third type are various combinations of exogenous and endogenous dormancy (Nikolaeva, 2001).

Most recently, Baskin and Baskin (2004a, 2014), proposed a modified version of Nikolaeva´s classification system splitting this into different classes and refers to mechanical dormancy as a part of physiological dormancy and considers chemical dormancy a limited condition in nature (Baskin and Baskin, 2003). This classification system consists of five principle dormancy classes (some classes contain sub-classes and levels), namely; physical (PY), physiological (PD), combinational (PY + PD), morphological (MD), and morphophysiological (MPD) (Baskin and Baskin, 2004a, b).

Furthermore, dormancy may also be classified based on the time of occurrence as either primary dormancy, which is present in mature seeds prior to and during dispersal, or secondary dormancy, which refers to dormancy that is imposed in mature seeds after imbibition under conditions that are unfavourable for initial germination. Indeed, seeds

with non deep physiological dormancy have been found to cycle in and out of dormancy which can regularly occur over many years (Bewley and Black, 1994; Vleeshouwers et al., 1995; Benech-Arnold et al., 2000; Baskin and Baskin, 2004a).

1.3.3.1 Physiological Dormancy

Physiological dormancy is particularly prevalent in species from dry arid lands (Baskin and Baskin, 2003; Erickson et al., 2016). Physiological dormancy is attributed to various inhibiting mechanisms within the seed that prohibit the growth and germination of the embryo such as covering structures that restrict radicle growth, inhibit oxygen access or contain chemical inhibitors (Baskin and Baskin, 1998). For example, in a study on *Panicum virgatum*, a perennial warm season grass, removing glumes and bracts and cutting the pericarp/testa as well as adjusting the oxygen concentration that seeds were exposed to resulted in high germination indicating that these structures may function as barriers to oxygen uptake (Duclos et al., 2013). The use of several temperature ranges over different time periods may affect the germination of seeds. Non dormant seeds germinate rapidly over a wide range of temperature, however this is not the case with dormant seeds as they need an optimum range of temperature to release dormancy. In nature seeds may have several cycles of dormancy and non-dormancy until favourite environment factors become available for germination (Geneve, 2003). A method to overcome physiological dormancy is by providing conditions that mimic local environmental characteristics such as dry afterripening (DAR) by storing seeds (6 months – 2 years) under warm dry conditions (20-35°C) (Merritt et al., 2007; Turner et al., 2009; Commander et al., 2009) or stratification by exposing seeds to moist cold or warm conditions (Turner et al., 2006; Merritt et al., 2007). In a study on *Lomandra preissii* warm stratification (26/13°C or 33/18°C) for several weeks was found to be useful to end dormancy (Merritt et al., 2007). An extended period of warm stratification (25/15°C) was found to release deep physiological dormancy in *Leptecophylla tameiameiae* seeds (Baskin et al., 2005). Cold stratification (4°C) for 3 months overcame dormancy in *Dipteronia dyeriana* seeds (Tang et al., 2012). Dry afterripening (warm dry temperature ≥15°C) is helpful in alleviating physiological dormancy especially in species with non-deep physiological dormancy from families such as the Asteraceae, Apiaceae, Brassicaceae, Solanaceae and Poaceae (Baskin and Baskin, 2004b; Iglesias-Fernández et al., 2011; Baskin and Baskin, 2014).

Indeed, many different studies indicate that afterripening can alleviate physiological dormancy in a large range of species which have shown that storage temperature, seed

moisture content and storage duration can affect the rate of dormancy loss (Turner et al., 2006; Merritt et al., 2007; Hoyle et al., 2008; Liu et al., 2013; Erickson et al., 2016)(Table1.1). Wet/dry cycling has also been found to be helpful in alleviating physiological dormancy which is a modification of dry afterripening conditions (Baker et al., 2005; Hoyle et al., 2008). For example, 10 to 20 weeks of dry/wet cycle alleviate physiological dormancy in *Goodenia cycloptera* (Hoyle et al., 2008) while Chia et al. (2016) found that wet/dry cycles were effective for dormancy loss in the deeply dormant seeds of *Persoonia longifolia.*

Scarification and seed nicking can also help overcome physiological seed dormancy in individual species which may be combined with heat or smoke to increase its effectiveness (Morris, 2000). The embryo covering layer can restrict the movement and concentration of oxygen to the embryo causing inhibition of germination (Baskin and Baskin, 2014). Scarification with acid can break dormancy and promote germination as well. Combinations of different treatments may lead to better results, for example, scarification with sulfuric acid (75%v/v) for 5 minutes followed by 60 days cold stratification increased germination of *Allium hirtifolium* to 87% (Dashti et al., 2012).

Table 1.1. A summary of treatments for overcoming physiological seed dormancy
Summary of main treatments successfully used for overcoming physiological seed dormancy under laboratory conditions.

Species	Family	Treatment summary	Authors
Actinobole uliginosum	Asteraceae	Wet/dry cycling	Hoyle et al. 2008
Anigozanthos manglesii	Haemodoraceae	Dry heat (100°C/3 hrs.)	Tieu et al. 2001
Austrostipa elegantissima	Poaceae	Dry afterripening + smoke	Turner et al. 2009
Begonia lithophila	Begoniaceae	Dry afterripening or moist chilling	Hu et al. 2012
Dipteronia dyeriana		Cold stratification	Tang et. al. 2012
Eremophila maculata	Scrophulariaceae (Syn. Myoporaceae)	Extraction of seeds from indehiscent endocarps	Richmond and Ghisalberti 1994
Goodenia fascicularis	Goodeniaceae	Warm stratification	Hoyle et al. 2008
Grevillea mucronulata	Proteaceae	Precision nicking + smoke	Morris 2000
Microlaena stipoides	Poaceae	Sulphuric acid exposure	Stevens et al. 2015
Prostanthera eurybioides	Lamiaceae	Dry heat (80°C/10min.), removal of mericarp plug from persistent calyx.	Ainsley et al. 2008
Triodia brizoides	Poaceae	Dry afterripening + Karrikinolide, removal of seeds from florets	Erickson et al. 2016

1.3.3.2 Physical dormancy

Seeds with physical dormancy possess a water-impermeable seed coat, which is commonly caused by the palisade layer within the outer seed or fruit coat. Physical dormancy occurs in at least in 18 families of angiosperm (Baskin and Baskin, 2014; Gama-Arachchige et al., 2013; Paulsen et al., 2014). It has been found that seeds with physical dormancy possess a small morpho-anatomical area in the seed coat called a "water gap" that regulates water uptake (Jayasuriya et al., 2015). Studies that examine treatments to break physical dormancy are based on increasing seed coat permeability to the water gap. Physical dormancy was alleviated in six species of common Australian Rhamnaceae species at the biodiverse of south-west Western Australia by using hot water treatment (88-92°C for 1 to 8 minutes) (Turner et al., 2005). Mechanical scarification and sulphuric scarification was found to be successful in alleviating PY in different Fabaceae species in the north west of the cold desert of China (Abudureheman et al., 2014). Exposure of *Cassia leptophylla* and *Senna macranthera* seeds from the Atlantic forest of Brazil to high temperatures (50°C for 4 hours) was successful for breaking the hard seed coat and opening water gap in both of these species (De Paula et al., 2012). Physical dormancy in

Dodonaea viscosa from Hawaii was alleviated by using several different methods; dry heat (80–160°C), dipping in boiling water for 5 to 15 seconds and mechanical scarification of seeds; all methods resulting in 100% germination (Baskin et al., 2004b). Exposure to concentrated sulphuric acid for up to 24 hours was found to be highly effective for dormancy loss in different species of Baobabs (*Adansonia* spp.) with PY (Turner and Dixon, 2009).

1.3.3.3 Combinational dormancy

Seeds with combinational dormancy contain both a hard impermeable-water coat and physiological dormancy (section 1.3.3.1). Treatments include alleviating physical dormancy as previously described (section 1.3.3.2) followed by the alleviation of physiological dormancy through afterripening or stratification (Baskin and Baskin, 1998). Combinational dormancy is considered as a double safety mechanism for preventing early germination in arid and Mediterranean regions, though it appears to be relatively uncommon (Assche and Vandelook, 2010).

In a study on *Sicyos angulatus* seeds, combinational dormancy was initially identified then overcome by firstly scarifying seeds then subjecting these to dry afterripening or cold stratification (Qu et al., 2012). Exposure to hot water for 15 seconds was found to alleviate PY in *Diplopeltis huegellii* seeds which were followed by dry afterripening for 6 to 12 months at 23°C to alleviate the PD component of the combinational dormancy that was present in the seeds of this species (Turner et al., 2006).

1.3.3.4 Morphological and morphophysiological dormancy

Seeds that contain morphological dormancy possess an embryo that is either underdeveloped but differentiated, or underdeveloped and undifferentiated (Baskin and Baskin, 1998). Embryos are typically very small relative to the size of the seed and endosperm and require growth and development before radicle emergence (Baskin and Baskin, 1998). Different environmental conditions are required to assist the embryo to grow inside the seed to a suitable size in order to germinate. However, it is common that seeds with morphological dormancy are also controlled by physiological dormancy which is referred to as morphophysiological dormancy. For example, in a study on *Aegopodium podagraria* the embryo within the seeds grew to six times their initial size, in about 8 weeks at 5°C but once reaching this size still did not germinate immediately requiring an additional 8 weeks at 5°C before germination occurred (Vandelook et al., 2009).

1.3.3.5 Dormancy key

Understanding the type of dormancy that may be present in the seeds is important between different species as it will guide and inform the development of treatments that are likely to result in successful germination. A dormancy classification key has been developed by Baskin and Baskin (2004a) and is presented in Table 1.2.

Table 1.2. Dichotomous key used for the classification of seed dormancy (Baskin and Baskin 2004a).

1. Seed/fruit coat not permeable to water, embryo fully developed..... 2

    2. Germination occurs within about 2 weeks (usually within a few days) when seed/fruit coat is scarified ..... **PHYSICAL DORMANCY**

    2. Germination does not occur within about 2 weeks (usually not even within a somewhat longer period of time) after seeds/fruit coat is scarified, although seed becomes fully imbibed within a few hours following scarification..... **COMBINATION OF PHYSICAL AND PHYSIOLOGICAL DORMANCY**

1. Seed/fruit coat permeable to water; embryo either fully developed or underdeveloped..... 3

    3. Embryo not differentiated, or if differentiated it is underdeveloped (small) 4

        4. Embryo not differentiated ..... **SPECIALIZED TYPE OF MORPHOLOGICAL DORMANCY**

        4. Embryo differentiated but underdeveloped (small) 5

            5. Embryos in freshly-matured seeds begin to grow (elongate) within a period of a few days to 1-2 weeks, and seeds germinate within about 30 days..... **MORPHOLOGICAL DORMANCY**

            5. Embryos in freshly-matured seeds do not begin to grow within a period of even a few weeks, and seeds do not germinate within 30 days ..... **MORPHOPHYSIOLOGICAL DORMANCY**

    3. Embryo differentiated and fully developed (elongated)..... 6

        6. Seeds do not germinate within about 30 days..... **PHYSIOLOGICAL DORMANCY**

        6. Seeds germinate within about 30 days..... **NONDORMANT**

1.4 The technical aspects of seed viability testing

It is important to consider three vital factors that affect seeds in seedbanks; initial seed viability, seed dormancy and seed ageing (Gooding et al., 2003). Mature seeds have a better germination potential, while immature seeds are much more sensitive to mould and disease, harder to process and may have delayed or weakened germination (Bainbridge, 2007). One of many ways to assure maturity and viability is by doing a seed viability test such as a cut test, x-ray assessment or tetrazolium test to assess seed fill or metabolic activity (Martyn et al., 2009). A cut test while slow and tedious is highly effective in determining the internal health of a seed and involves cutting seeds into sections and carefully examining their internal structure. Seeds that have white firm endosperm or other tissues are considered healthy while seeds that are empty, squishy, or brown/grey are likely to be non-viable. The biggest disadvantages of this technique are that it is destructive, and slow. X-ray analysis is relatively new and provides a fast non-destructive test by producing an image of the inside of the seed showing its internal structure highlighting which seeds are filled, damaged or shrivelled (Kodym et al., 2010). Seeds considered to be in good health can still be used as they are not harmed or damaged in any way. The biggest disadvantage is obtaining access to an x-ray machine as these are considerably expensive. The third major seed test is tetrazolium staining which indicates the viability of the seeds by turning to a pink or red colour based on metabolic activity within the seed (Lakon, 1949). The internal tissues of seeds that are metabolically active turn bright pink in the presence of tetrazolium while dead or damaged tissues with no or low levels of metabolic activity do not change colour at all. The tetrazolium test can be highly variable and is somewhat subjective. It is also tedious and slow and does not provide information as to whether the seed will actually germinate if found to be alive (Martyn et al., 2009). However, even after conducting different viability tests the most trusted method to determine seed quality and viability is to germinate a representative sample (Baskin and Baskin, 1998). Other important tests can assess seeds size, hardness and seed moisture content which also provide useful information (Bainbridge, 2007; Erickson et al., 2016b).

1.5 Enhancement of seed germination through priming in arid regions

In the Pilbara region of Western Australia, the climate is semi-arid and receives ~ 300 mm low irregular rainfall annually; mainly during the summer months following tropical cyclonic events. Annual and ephemeral species germinate rapidly and start

growing and reproducing quickly after rainfall (Department of Industrial Development, 1983, Erickson et al., 2016b). Perennial grasses are considered as autumn species as they respond to summer rainfall and start vegetative growth and flowering (Jan-Mar) after sufficient rain and then rapidly dehisce seeds during autumn after 4 to 6 weeks of flowering (Erickson et al., 2016b) (Figure 3.1). The timing of rainfall is critical as water represents the principle factor regulating all phenological events including vegetative growth, flowering and seed germination (Beatley, 1974; Anderson and Ostler, 2002). For example, Ghazanfar (1997) found that late rains delayed all phenological phases in the annual and perennial species occurring in a gravel desert wadi in northern Oman. In a study on five native species from the Pilbara it was found that emergence depends on adequate soil moisture as a prime factor tightly regulating germination (Muñoz-Rojas et al., 2016).

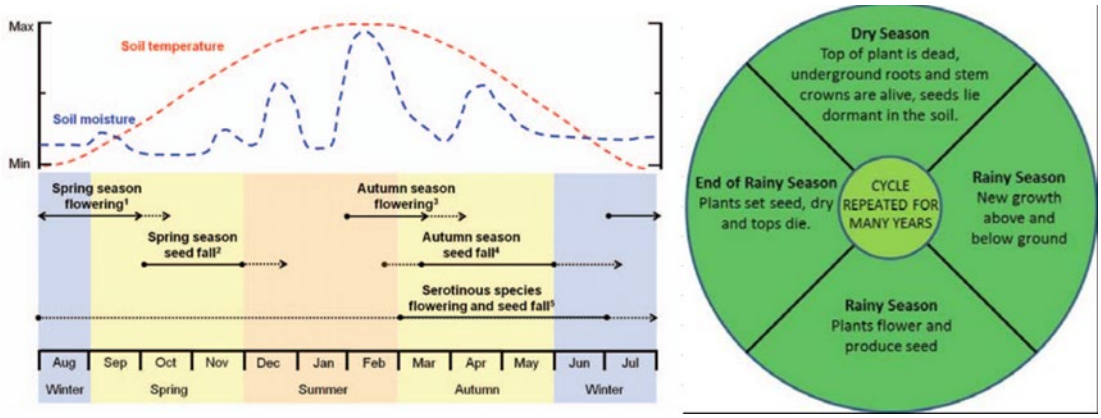

Figure 1.5. Pilbara flowering season (left); generalised plant life cycle (right)

The flowering season (left) in the Pilbara region of Western Australia (Erickson et. al., 2016b), and a generalised life cycle (right) of arid-dwelling perennial plants (Melvin George and Kevin Rice rangelandarchive.ucdavis.edu/Publication)

When rain does occur in arid regions it can be intense though brief with significant amounts of moisture lost through runoff and evaporation (Pilgrim et al., 1988). About 90-95% of water evaporates in the upper of 5-10 cm of the soil following a rainfall event especially during the hot summer months (Ritchie and Johnson, 1990) which exposes the majority of seeds that lay in this level to rapid wetting and drying cycles. Studies illustrate that this rapid wet-dry cycling can break dormancy in some seeds that contain physiological dormancy (Hoyle et al., 2008a; Chia et al., 2016), whereas in non-dormant seeds it may “prime” these seeds and change their germination attributes if the soil

moisture does not persist for a sufficient amount of time (Bewley and Black, 1982). For example, in a study on perennial ryegrass (Lolium perenne L.) and annual bluegrass (Poa annua var. annua) seeds exposed to different wet/dry cycles showed a delay in germination but improvement in germination uniformity (Allen et al., 1993). In contrast, some studies show that wet/dry cycles can also stimulate seed germination and enhance germination speed in a similar manner as agricultural seed priming (Gonzalez-Zertuche et al., 2001).Seed priming is an effective method for enhancing germination in arid and semi- arid environments as it can improve water uptake efficiency (Halmer, 2000; McDonald, 2000). The concept of seed priming is to improve seed germination effectiveness, the rate of emergence as well as germination synchronicity under stressful environmental conditions (Chen et al., 2010). In addition, it may also improve seedling vigor, but as a downside it may negatively impact seed longevity and its beneficial effects may not persist when primed seeds are stored for any significant length of time (Butler et al., 2009; Varier et al., 2010). The method consists of imbibing seeds in different solutions (including water or osmotica) and in their hydrated state metabolic processes begin to occur in the seeds but are stopped before reaching the point of radicle emergence by removal from the solution and drying back to a low moisture content (Halmer, 2000; McDonald, 2000; Jisha et al., 2013, Paparella et al., 2015). Seeds can then be sown at a later stage as normal with the beneficial effects derived from the priming treatment manifesting themselves as improved seed performance (Halmer, 2000; McDonald, 2000; Jisha et al., 2013, Paparella et al., 2015). There are several types of priming methods. One of the simplest and oldest methods is hydropriming, which basically is soaking seeds in water. Hydro priming was developed for several priming methods such as osmopriming which exposes seeds to low water potential and solid matrix which is considered similar to the previous method but is environment friendly as it uses solid organic and non- organic material. Other methods of priming use microorganisms and bioactive molecules, such as biopriming and chemopriming which is mainly used to prevent microbial infections (McDonald, 2000; Zhang et al., 2007; Olszewski et.al, 2012; Paparella et al., 2015; Mahmood et al. 2016).

1.6 Conservation and restoration of plants in arid lands

1.6.1 Botanic garden concept in plant conservation

In dryland regions, the main challenge in restoration involves turning the desertification process around by capturing and holding water in order to revive the land

(Bainbridge, 2007). It is important to consider plants when protecting biodiversity and iconic landscapes and to aid soil improvement as their presence can increase soil moisture because vegetation can capture and retain sporadic rainfall. Plants also provide habitat for other species including other species of plants as well as microbes, fungi and animals living above the surface and below the ground (Bainbridge, 2007). One of the oldest ways to protect and conserve natural areas and resources was by restricting access to certain lands which saved many species from extinction (Leadlay and Jury, 2006). The concept of establishing botanic gardens in arid lands while novel is significant, as these are a relatively recent development although there is little information available about implementing and maintaining botanic gardens in arid environments (Sellers, 1988). In addition, worldwide, many native plants are rare and at risk of extinction, with one of the main threats currently faced by many different species being habitat loss due to agriculture, mining, urbanization and industry pressure (Oldfield, 2010). Botanic gardens are critical to ensure that rare and threatened species are secured as they provide technical expertise in the propagation and horticulture of these species as well as the facilities to hold them for extended periods of time while in situ restoration work may be implemented.

One of the main functions of botanic gardens is to document and display the collection of living plants that reflect certain environments for the purposes of scientific research, conservation, display and education (Wyse Jackson and Sutherland, 2000). Herbariums which are collections of preserved plant (and fungi) specimens are often placed within botanic gardens and document the identity and location of plants for taxonomic research. Botanic gardens with large internationally significant herbaria include the Royal Botanic Garden Sydney, Missouri Botanic Garden, and the Royal Botanic Garden Kew.

Plants conservation is a major role for botanic gardens and assists in the maintenance of plant diversity around the world (Moskwa and Crilley, 2012). The existence of botanic gardens provides much more value than simply being a beautiful place to visit because as botanic gardens can improve human welfare in a number of important ways so there are many reasons for establishing botanical gardens with different structures and activities to suit local needs and conservation agendas (Waylen, 2006). One function of botanic gardens is to protect plants from extinction through *ex situ* seed banking. The Royal Botanic Gardens, Kew, in the United Kingdom maintains one of the largest ex-situ seed banking programs in the world, which conserves over 13% of the world's wild plant species at present and aims to save 25% of the world’s flora by 2020 using seed banking methodologies (Sharrock et al., 2010).

In many countries botanic gardens are used to display medicinal, agricultural and other plants of economic importance and are also centres for scientific research, education (Heywood, 2010) and medicine (Forbes, 2008). Only a relatively few botanic gardens take an active role to fight plant extinction and the loss of biological diversity (Powledge, 2011) with scientific research generally limited when compared to the resources devoted to the other roles of botanic gardens, such as horticulture and taxonomy (Furse-Roberts, 2005; Moskwa and Crilley, 2012).

1.6.2 Botanic garden concept in land restoration

Restoration projects are important as it may take a long time for natural processes to rehabilitate damaged environments with outcomes often not aligned with conservation goals (Bainbridge, 1990) (Figure 1.5). The role of botanic gardens in restoration may provide different aspects unavailable from other organisations such as propagation expertise and access to research outcomes involving relevant species or ecosystems. Botanic gardens can also provide the scientific experience to successfully conduct restoration research under *ex situ* and *in situ* conditions, which may lead to improvements in current restoration approaches (Hardwick et al., 2011).

Seeds in desert ecosystems are not easy to find because they are rare, or occur in isolated and hostile locations or may only be found in abundance every few years after infrequent and sporadic rainfall events (Erickson et al., 2016b, 2017). Also, the quality (e.g. viability) of seeds produced by plants can be different each year due to the difference in temperature, moisture and winds, so obtaining sufficient quantity of viable seeds for restoration may not be possible without a concerted effort of seed collection and banking over many years (Gooding et al., 2003). In addition, seeds of most species in arid lands are likely to be dormant when collected, and may require pre-treatments to alleviate dormancy before use in restoration (Bainbridge, 2007).

It is essential in restoration to understand the type of plants that can be used in the target location and this can be done through collecting information on different plant and seed characteristics (Erickson et al., 2016). For a species to be used in restoration sufficient seeds need to be collected, processed and stored for *ex situ* plant propagation (including research) and *in situ* direct seeding (Burke, 2003). Botanic gardens can guide habitat restoration through spatially referenced biological collections and through providing DNA in order to understand genetic diversity and issues of provenance (Leadlay and Jury, 2006; Krauss and He, 2006).

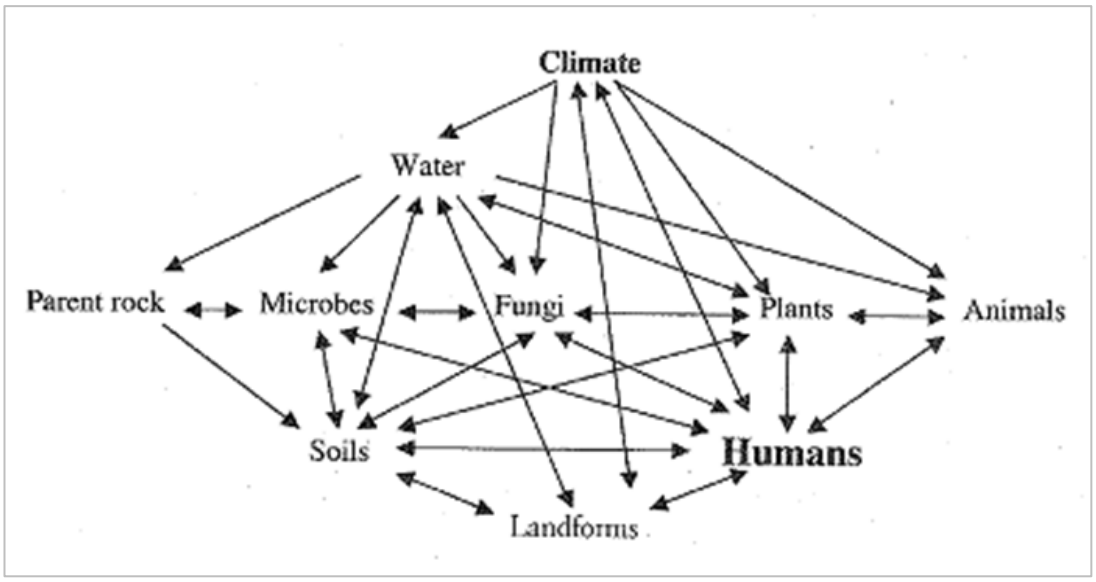

Figure 1.6. Factors (including humans) that affect landscape processes and how these interact with ecosystem structure (Bainbridge, 2007).

1.7 Research objectives

1.7.1 General objective

This research aims to investigate mechanisms to overcome seed dormancy and improve germination of plants endemic to arid environments in the Pilbara region of Western Australia; Poaceae, Cyperaceae and Goodeniaceae species that exhibit inherent seed dormancy and problematic germination and the attributes of botanic gardens that assist in the conservation of plant species.

1.7.2 Specific objectives

1. To develop reliable and repeatable methods for seed-based propagation by classifying dormancy types in representative Cyperaceae, Goodeniaceae and Poaceae species from arid environments.
2. To identify factors responsible for dormancy break (afteripening, wet/dry treatments) and subsequent germination in representative Cyperaceae, Goodeniaceae and Poaceae species from arid environments.
3. To investigate seed priming techniques as a method to enhance germination in representative Poaceae species from arid environments.

4. To improve the conservation and exhibition of plants in an arid botanical garden from a range of phytogeographic zones by assessing how their functionality can be improved..

Chapter 2 Seed biology of selected herbaceous perennial species required for mine site restoration in a semi-arid environment

2.1 Abstract

Restoration of arid lands is essential as many have been disturbed by human activities across the globe. Seeds are an important component of land restoration however, a significant proportion of species from arid regions possess seeds with different dormancy and germination requirements that do not readily emerge when sown *in situ*. This study aimed to determine the general seed biology and germination capacity of seven understory species from three families (Cyperaceae, Goodeniaceae, and Poaceae) that are endemic to the semi-arid Pilbara region of Western Australia and necessary for successful mine-site restoration and then assess different dormancy breaking treatments. Seed viability, imbibition capacity and seed mass were initially assessed then a subset of seeds were incubated at a range of temperatures (20, 25, 30, 35, 20/35, 40, 25/40, 25/45, 50, 25/50°C) on different media (H_2O, GA_3, KAR_1) to assess their germination capacity. All seeds were found to have high viability and to readily imbibe water, however the seeds of *Eragrostis eriopoda, Fimbristylis dichotoma* and *Goodenia stobbsiana* were also observed to have low initial germination so had physiological dormancy. In comparison, the seeds of *Cymbopogon obtectus*, *Eriachne mucronata*, *Goodenia armitiana and Goodenia cusackiana* were found to be largely non-dormant and easy to germinate. The optimal germination temperature for the seeds of *Eragrostis eriopoda* and *Fimbristylis dichotoma* was very high (~40°C), while for *Goodenia* spp. lower incubation temperatures (~25°C) were found to be more effective. To assess afterripening, seeds from selected species were placed under different storage conditions with a subset regularly exposed to a brief pulse of moisture to simulate intermittent thunderstorms that are common to the Pilbara region. The mechanical scarification treatment was applied using a pneumatic seed scarifier while acid scarified seeds were treated with 50% (v/v) H_2SO_4. Afterripening seeds at 50°C and 50 % RH for 26 weeks increased germination in *Eragrostis eriopoda* to ~30% when incubated at 25/45°C, while wet/dry cycling at 30°C and 50% RH for 18 months improved germination of *Fimbristylis dichotoma* seeds to >50% when incubated at 40°C. Afterripening at 30°C and 50% RH for 8 months was found to increase germination of

Goodenia stobbsiana to 65% when incubated at 25°C in the presence of KAR_1. Acid scarification of *Eragrostis eriopoda* seeds for 60 minutes and incubation on KAR_1 at 40°C result in ~16% germination, while a similar approach for *Fimbristylis dichotoma* seeds resulted in 47% germination when these were incubated at 25/40°C. This study obtained high (>70%) germination for four out of the seven species assessed. For the three physiologically dormant taxa (*Eragrostis eriopoda*, *Fimbristylis dichotoma* and *Goodenia stobbsiana*), several dormancy breaking treatments were proven to be partially effective, thus revealing possible solutions for reliably breaking dormancy that will assist in future restoration programs.

2.2 Introduction

One of the largest biogeographical regions in Western Australia is the Pilbara, located in the north west of Western Australia; bordered by the Indian Ocean to the west and the Great Sandy Desert to the east (Figure 2.1). According to the Interim Biogeographic Regionalisation for Australia (IBRA) classification scheme (Thackway and Cresswell, 1995), the Pilbara is comprised of four sub-regions; Hamersley, Fortescue, Chichester, and Roebourne (Figure 2.1).

The Pilbara region is one of the oldest landscapes in the world with some rock formations dated to over 3.5 billion years old and containing some of the oldest signs of life ever discovered (Haslam McKenzie, 2013; Djokic *et al*., 2017). The Hamersley sub-region covers about 6,215,092 ha and is located in the southern section of the Pilbara craton and is described as a rugged landscape of Proterozoic sedimentary ranges and plateaus divided by highly weathered gorges (Kendrick, 2001b). The Fortescue sub-region covers 2,041,914 ha and is centred on the Fortescue River and associated drainage systems (George and McKenzie, 2015). It is characterised largely as alluvial plains and river frontage, punctuated with extensive salt marshes (Kendrick, 2001a). The Chichester sub-region located in the northern section of the Pilbara craton is described as consisting mainly of undulating Archaean granite and basalt plains covering about 9,044,560 ha (Kendrick and McKenzie, 2001). The smallest and fourth subregion is the Roebourne located along the coast in the northern and the north-western part of the Pilbara (George and McKenzie, 2015) and consists of Quaternary alluvial and older coastal and sub-coastal plains which covers 2,008,983 ha (Kendrick and Stanley, 2001).

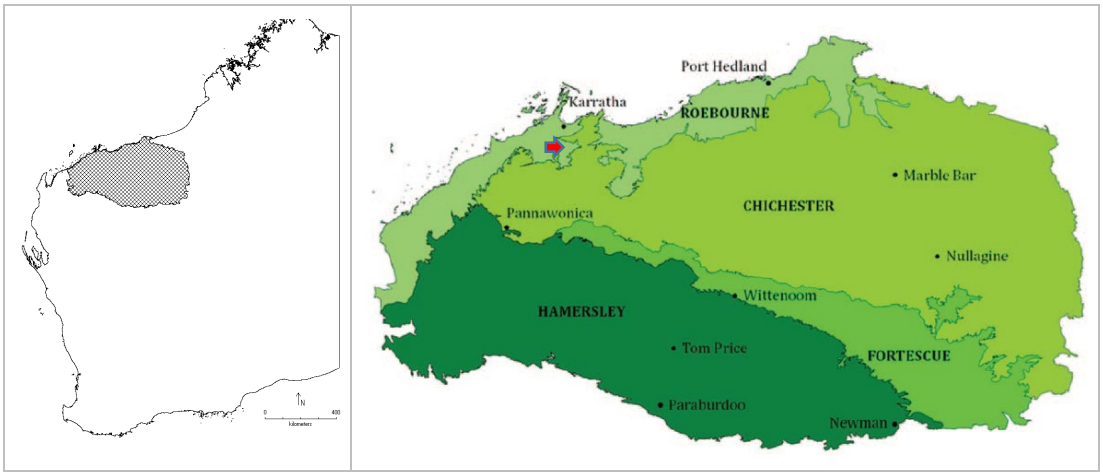

Figure 2.1. The Pilbara region and four subregions showing the site where seeds are collected (red marker).
The Pilbara (shaded) region located in the north of Western Australia (left image) contains four subregions (right image): Hamersley, Fortescue, Chichester, and Roebourne (Erickson et al., 2016).

The climate of the Pilbara is semi-arid and tropical experiencing occasional rainfall (~300 mm annually) mainly during the summer period due to monsoonal activity that causes regular thunderstorms and occasional cyclonic events with extreme (>100 mm) rainfall conditions. Temperatures during summer are high to very high regularly exceeding 40-45°C while in winter it is around 20°C across the region (Kendrick and Stanley, 2001; Van Vreeswyk et al., 2004) (Figure 2.2). The broad diversity in geology and localised climatic conditions results in a rich regional habitat and vegetation (Pepper et.al, 2013, George et.al, 2015). There are approximately 1,800 plant species with the largest most dominant families being the Fabaceae (330 species), Poaceae (227 species), Malvaceae (142 species) and the Asteraceae (142 species) (Erickson et. al, 2016; Western Australian Herbarium, 1998). Spinifex (*Triodia* spp.) which are a type of grass are particularly dominant as well as diverse and adapted to the poorest soils, they grow across wide coastal plains alongside other grasses such as *Eriachne* spp. and *Eragrostis* spp. In deeper soils and along horizontal drainage lines, *Acacia* shrublands are found with occasional *Eucalyptus* trees occupying rocky hills and slopes. Following sufficient rains, many different annual and ephemeral species can be found which grow and reproduce rapidly taking advantage of the brief pulse of moisture and warm to hot growing conditions (Department of Industrial Development, 1983). In the Pilbara, plants that have root systems near the soil surface such as many herbaceous perennial species, start actively growing after rainfall (between January and March) rapidly flowering and seeding within 4-6 weeks. In comparison, woody trees and shrubs with much larger root systems access water much deeper in the soil profile, flowering between July and September with seed

production and maturation occurring shortly thereafter, between September and December (Erickson, 2016; Richie *et al.* 2017).

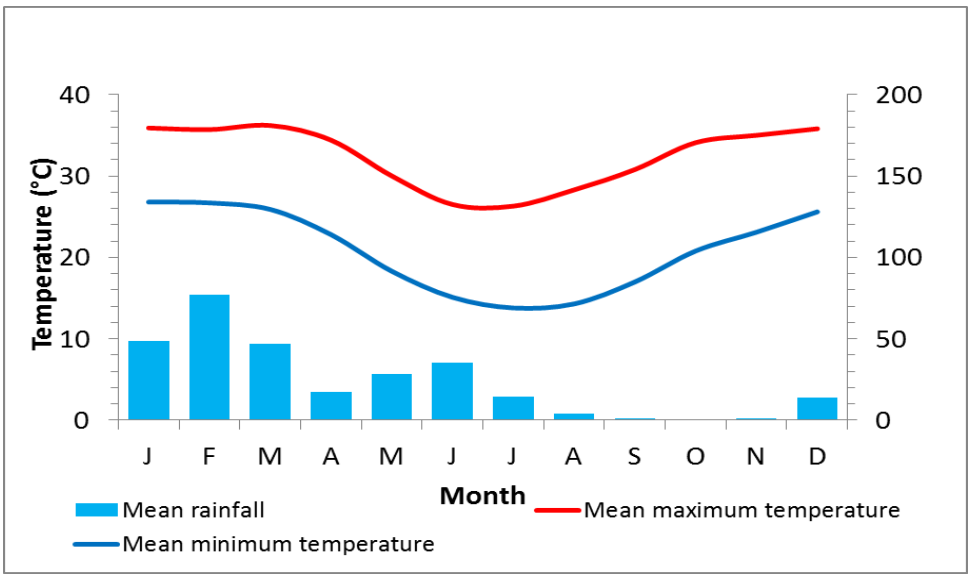

Figure 2.2. Mean monthly climate for Newman in the Pilbara region of Western Australia. *Shown in this figure are mean maximum and minimum temperatures (°C) for years 1993 to 2018 and mean monthly rainfall (mm) for years 1972 to 2018. Two distinct seasons are recognized in the Pilbara, the wet season which runs from December to the end of June and a dry season running from July until November (Bureau of Meteorology, 2018).*

Dominant land uses for the Pilbara are grazing, managed Aboriginal land, conservation and mining (Department of Planning, 2009). Mining is very important to the regional economy and in 2012-13 the total value of mineral (such as iron ore, gold) and petroleum in the Pilbara was over $70 billion, representing 72% of the value of mineral and energy production in Western Australia (DRD, 2015). However, mining is locally destructive leading to significant disturbance of natural ecosystems through the removal of soil and vegetation and by the burial or stockpiling of different types of waste (Cooke and Johnson, 2002). Consequently, the mining industry has a significant obligation to undertake the rehabilitation of disturbed lands (Risbey, 2016).

Challenges in restoration ecology include having sufficient amount of seeds (Merritt and Dixon, 2011) for effective land restoration when covering large scale areas in arid drylands (Wagner et al., 2008; Kildisheva et al., 2016). Seed amounts available for restoration are limited by other factors as well such as poor seed viability, limited germination capacity due to seed dormancy and a high proportion of empty florets (Erickson et al, 2016). At present knowledge on how to resolve these traits is lacking for

a large number of wild species with ~65% of Pilbara species for example, possessing some form of seed dormancy making these difficult to reliably germinate without prior knowledge (Erickson et al., 2017). Successful site restoration may be achieved through choosing native plants that are found locally in the Pilbara with three of the dominant families, namely the Poaceae (227 spp.), Cyperaceae (63 spp.) and Goodeniaceae (61 spp.) being both important elements of the understory and in many cases displaying problematic and erratic germination and seedling emergence under both laboratory and field conditions (Erickson et al., 2016).

Low germination percentage and dormancy is a common problem with grass species such as *Sorghum timorense*, *Triodia* spp. and *Neurachne muelleri* (Waters et al., 1997; Farley et al., 2013; Erickson et al, 2016). Physiological dormancy is the most common class of seed dormancy in dry arid regions (Baskin and Baskin, 2004a; Commander et al., 2017; Erickson et al., 2017) and is linked to various inhibiting mechanisms inside the seed that constrain the growth and germination of the embryo such as covering structures that restrict radicle growth, inhibit oxygen permeation or contain chemical inhibitors (Mott, 1972; Farley et al., 2013; Baskin and Baskin, 2014).

In arid regions, grass species that have physiological dormancy are commonly alleviated during the warm season mainly by dry afterripening (Merritt et al., 2007). Temperature is considered the primary factor affecting dormancy status which interacts with relative humidity to alter the seed moisture content (Vleeshouwers and Bouwmeester, 2001). Simulating these environmental conditions to release dormancy through afterripening and dry/wet cycling can be useful when seed germination stimulants such as gibberellic acid (GA_3) and karrikinolide (KAR_1) are ineffective for overcoming dormancy and promoting germination. For example, *Eragrostis eriopoda* does not germinate under a range of conditions or when exposed to stimulants such as GA_3 or KAR_1 (Commander et al., 2017). Dry afterripening was found to release dormancy in *Triodia* species (Poaceae) when stored at 30°C/50 % RH after 6-24 months in both florets and seeds (Erickson et al., 2016). Manipulation of the seed storage environment from 1-18 months affected the rate of dormancy loss in the seeds of *Anthocercis littorea*, *Dioscorea hastifolia* and *Zygophyllum fruticulosum* with all three species showing improved germination over time (Commander et al., 2009). High levels of germination were observed in the seeds of *Goodenia cycloptera* and *Velleia glabrata* (Goodeniaceae) after treatment with dry/wet cycling which proved to be more effective in releasing dormancy than dry afterripening (Hoyle et al., 2008). One of the causes of physiological dormancy may be due to inhibiting

mechanisms in the seed that restricts the growth of the embryo due to the covering seed layers. In these cases, scarification of the coat may assist in releasing dormancy and consequently promotes germination (Finch-Savage and Leubner-Metzger, 2006). As an example, using sulfuric acid has been found to release coat-imposed dormancy in the seeds *Capparis spinosa* after seeds were soaked in concentrated sulfuric acid for 20 minutes (Sozzi and Chiesa, 1995).

Seeds germination traits of perennial understory species such as those from Poaceae, Cyperaceae and Goodeniaceae has not as yet been sufficiently addressed so these groups remain largely problematic in terms of their reliable use in restoration programs (Merritt et al, 2007; Erickson et al, 2016; Commander et al., 2017). This study aimed to investigate the seed biology of seven common native species from the Pilbara region in the north west of Western Australia, namely the Poaceae species: *Cymbopogon obtectus* S.T.Blake, *Eriachne mucronata* R.Br., and *Eragrostis eriopoda* Benth.; the Cyperaceae species *Fimbristylis dichotoma* Vahl. and; the Goodeniaceae species *Goodenia stobbsiana* F.Muell., *Goodenia armitiana* F.Muell. and *Goodenia cusackiana* (F.Muell.) Carolin. These were selected as representatives of families with three distinctive understory lifeforms (grasses, forbs and shrub), and comprise species for which relatively little information is known regarding seed dormancy or germination characteristics. For each species, the following attributes were investigated: (a) seed and embryo characteristics; (b) patterns of water uptake (i.e. imbibition); (c) germination in response to four different incubation temperatures; (d) germination in response to the application of different germination stimulants (water, gibberellic acid (GA_3) and karrikinolide (KAR_1)); and (e) different dormancy alleviating approaches such as afterripening, wet/dry cycling, mechanical scarification and acid scarification for species that were confirmed from earlier experiments to have physiologically dormant seeds.

2.3 Materials and Methods

2.3.1 Seed collection

Seeds were collected from selected sites in the Pilbara, Western Australia (Figure 2.1). Collections were made between 2011-2015 (Table 2.1). Following collection all seeds were removed from their fruits or florets (where present), then air dried and stored in a refrigerated cool room for several months (~5°C and 50% RH). Seeds were sent after this time to Kings Park for storage in a seed drying room (15°C and 15% RH) with

germination testing commencing in November 2015 for the Poaceae and Cyperaceae species, and in 2017 for the three Goodeniaceae species. Distribution map of the location of the recorded species are displayed in Appendix A.

Table 2.1. Summary of the collection details for the species used in the study.

Family	Scientific name	Species attributes	Place of collection	Location coordinates	Date of collection
Poaceae	*Cymbopogon obtectus*	Tufted perennial grass to 1 m high	Mulga Down	22°19'12" S 118°42'53" E	March 2015
Poaceae	*Eriachne mucronata*	Caespitose, wiry perennial grass to 0.8 m high	Wittenoom	22°15'45" S 118°17'28" E	Sept/Oct 2014
Poaceae	*Eragrostis eriopoda*	Caespitose, perennial grass to 0.6 m high	Mulga Down	22°17'34" S 118°44'40" E	April 2015
Cyperaceae	*Fimbristylis dichotoma*	Shortly rhizomatous, tufted perennial sedge to 0.6 m high	Marillana	22°39'13" S 119°39'36" E	March/April 2015
Goodeniaceae	*Goodenia stobbsiana*	Prostrate or erect, much branched, viscid shrub to 1 m high	Marillana	22°28'35" S 119°46'28" E	Oct 2011
Goodeniaceae	*Goodenia armitiana*	Erect, densely tufted perennial herb to 0.5 m high	South of Telfer	22°57'59" S 122°31'51" E	Oct 2011
Goodeniaceae	*Goodenia cusackiana*	Erect top spreading, semi-woody perennial herb to 0.6 m high	Wittenoom	22°15'00" S 118°16'48" E	Oct 2011

2.3.2 Seed and embryo characteristics

Seeds were dissected under a stereo microscope in order to observe and classify the embryo type according to Martin (1946) and Baskin and Baskin (2007). At the same time the presence or absence of endosperm was also recorded. Seed fill was assessed by using a Faxitron MX-20 Digital X-ray cabinet (Faxitron, Tucson, AZ, USA; Kodym et al. 2010) on four replicates of 100 seeds, with seeds containing white/grey internal structure considered as filled (Figure 2.3). Seed viability was also estimated using two different approaches; a cut test and tetrazolium test. For the cut test, three replicates of 20 imbibed seeds were cut in half and then carefully assessed under a binocular microscope. Seeds were considered to be healthy if they had a firm, fully-formed, white endosperm and

embryo. In comparison, seeds that were shrivelled and appeared black/grey/off white internally were considered non-viable. Tetrazolium chloride staining was also used to test metabolic activity by firstly removing the seed coat and placing seeds onto filter papers containing 1% (w/v) tetrazolium chloride; the seeds were then incubated in darkness at 30°C for 24 hours. After this time, the seeds were removed, dissected to expose the embryo, and then carefully examined under a binocular microscope to determine whether the embryo had changed to dark pink in the presence of tetrazolium (indicating a viable seed). Average seed weight (mg/seed) was estimated by weighing three replicates of 100 seeds for each species. Average seed length (µm/seed) was recorded by using three replicates of 20 seeds measured under a Leica dissecting microscope fitted with an ocular graticule. Embryo: seed (E:S) ratio was calculated using the following formula: E:S= embryo length / seed length. Embryo type and E:S ratios were used to determine if seeds had a fully developed or underdeveloped embryo.

2.3.3 Seed coat permeability to water

For all species, seed coat permeability was assessed through an imbibition test by using three replicates of 100 seeds (>0.03 g/replicate). Seeds were assessed to obtain an initial weight then placed on moistened filter paper (Advantec, 84 mm) in 90 mm Petri dishes and incubated under standard laboratory conditions (~23°C) for several days. Seeds were regularly removed from Petri dishes, patted dry to remove excess moisture and re-weighed. This was done after 1, 2, 4, 6, 24, 48 and 72 hours of moist incubation.

Percentage water uptake over time was then calculated with the amount of absorbed water determined by the actual increase in mass of the florets/seeds with the results converted to percentages using (Equation 1) described by Orozco-Segovia et al. (2007):

Equation 1.

$$\%\ increase\ in\ mass\ = \left[\frac{(mass\ of\ imbibed\ -\ dry\ seeds)}{dry\ seeds}\right] * 100 \quad \textbf{(1)}$$

2.3.4 Germination test

Seeds of all seven species were used in the first germination experiment (Table 2.1). For all germination experiments, seeds (4 replicates of 25 seeds/plate) were surfaced sterilized (as a pre-caution procedure to prevent fungal disease) in 2% (w/v) calcium hypochlorite ($Ca[OCl]_2$) solution under intermittent vacuum for 30 minutes (10 minutes on-off-on, at -70kpa) and rinsed four times in sterilized de-ionized water in a laminar flow cabinet. Seeds of *Cymbopogon obtectus, Eriachne mucronata* and *Eragrostis eriopoda* were plated with and without florets; *Fimbristylis dichotoma, Goodenia stobbsiana, Goodenia cusackiana and Goodenia armitiana* were plated as seeds (i.e. no covering structures) onto Petri dishes containing 0.7% (w/v) water agar only, or Petri dishes containing water agar with 2.89 mM GA_3 (Gibberellic acid, Sigma-Aldrich Co., Australia), or 0.67 µM KAR_1 (Karrikinolide - 3-methyl-2H-furo[2,3-c] pyran-2-one), which was synthesized following the process described by Flematti et al. (2005). These concentrations were chosen as they were found to be successful in previous studies (Roche et al., 1997; Adkins and Peters, 2001; Flematti et al., 2005; Turner et al., 2009; Commander et al., 2009; Cross et al., 2013). In order to prevent moisture loss once seeds were placed onto the different media, Petri dishes were wrapped with plastic film. Seeds/florets were then incubated at a range of temperatures (20, 25, 30, 35, 20/35, 40, 25/40, 25/45, 50, 25/50°C) which were chosen to be temperatures representative of the Pilbara following rainfall events, with the response of seeds trialled in preliminary tests. The incubator (Thermoline Scientific) was set to a 12/12 hour alternate light and dark cycle (white fluorescent tubes, 30 W, with a photon flux density of ~30 µmol m-2 s-1, 400-700 nm) with seeds left exposed to these conditions. Seeds were scored weekly for up to 35 days (some of the seeds were left for longer duration with no significant difference in germination) with germination defined as radicle emergence greater than one-third of the seed length.

2.3.5 Effect of afterripening of seed germination

In the first experiment seeds of two species were used, *Eragrostis eriopoda* and *Fimbristylis dichotoma*. Florets and seeds were placed under standard dry afterripening conditions (Turner et al. 2009b). This environment was achieved by using an incubator (Contherm plant growth chamber, CAT 620 RHS) set to constant 30°C and 50% RH for the duration of the afterripening experiment. Seeds were removed after certain times (1, 3, 9, 12, and 18 months) and tested for germination using the same germination methods previously described (section 2.3.4) with seeds incubated at 25°C on either water only

or 0.67 µM KAR_1. Additionally, *Fimbristylis dichotoma* seeds were also incubated after 18 months of after-ripening at 40°C.

Eragrostis eriopoda seeds were also after ripened for 26 weeks at different afterripening temperatures (40°C, 50°C, 60°C) and 50% RH then plated onto Petri dishes containing either H_2O or KAR_1 (as previously described) and exposed to one of three different incubation temperatures, namely; 30°C, 20/35°C, or 25/45°C.

In the second experiment, the effects of dry afterripening were examined in seeds of three *Goodenia* species; *Goodenia stobbsiana, Goodenia cusackiana* and *Goodenia armitiana* by using the same incubator, temperature and humidity described in the first experiment (30°C and 50% RH). Seeds were removed after 1, 3, 6 and 8 months and germination was tested using the same germination method previously described (section 2.3.4) with seed incubated at 25°C, 35°C, 20/35°C, or 25/45°C on either H_2O or 0.67 µM KAR_1.

2.3.6 Effect of wet/dry cycling on seed germination

Seeds of two species only were used in this experiment, namely; *Eragrostis eriopoda* and *Fimbristylis dichotoma* due to sufficient quantity of seed available using the same storage conditions (30°C and 50% RH) as previously described. In addition to this after-ripening treatment, some seed treatments in this experiment were also regularly soaked in water for 24 hours once per fortnight then removed from the water and returned back to the incubator to dry. The process was repeated until seeds were removed and assessed for germination after 1, 3, 9, 12, and 18 months using the same approach as previously described (section 2.3.4). In addition, for *Fimbristylis dichotoma* seeds a sub sample was also incubated at 40°C to assess germination capacity after 18 months storage. This higher temperature was included because it is endemic to the Pilbara region were summer temperature exceed 40°C.

The same wet/dry experimental procedure was used for seeds of *Goodenia stobbsiana, Goodenia cusackiana, Goodenia armitiana* which were maintained under these conditions for up to 8 months. As previously described, seeds were removed and assessed for germination using the same methodology and temperatures on either water only or 0.67 µM KAR_1.

2.3.7 Effect of chemical and mechanical scarification on seed germination

Chemical scarification was assessed by using dilute (50% v/v) sulfuric acid (95-98% ACS reagent grade H_2SO_4, Sigma-Aldrich, St Louis, USA). The procedure was conducted by preparing an ice bath in the fume hood. Sulphuric acid was added in small volumes into the flask containing de-ionized waterand waiting until the solution cooled off (50 ml of H_2SO_4 added to 50 ml of de-ionized water) and then applied for two different times (30 and 60 minutes) for both species.

Following application, the sulfuric acid was removed using a disposable pipette and replaced instantly with a neutralising solution (8.4 g L^{-1} $NaHCO_3$ Sigma- Aldrich, St Louis, USA) which was then removed after five minutes by pipette with the remaining seeds rinsed several times with tap water. Treated and washed seeds were transferred to a seed drying room (15%RH and 15°C) for three days before being assessed for germination using the same methodology as previously described and exposing the seeds to a range of incubation temperatures (i.e. 25, 35, 40, 20/35, 25/40, 25/45°C).

Mechanical scarification was assessed by using a pneumatic seed scarifier (Model PSS2000, OEM, Inc. Corvallis, Oregon, USA) on a subsamples of seeds (Figure 2.4). This was done by blowing seeds around different grades of sandpapers for different periods of time. *Eragrostis eriopoda* and *Fimbristylis dichotoma* species were mechanically scarified for five different time periods (i.e. 30 seconds, 1, 2, 5, and 10 minutes) with 30 psi air pressure. In addition to these different exposure times, four grades of commercially available (3M, North Ryde, NSW, Australia) abrasive paper (P180 (fine), P120, P80, and P60 (coarse)) were assessed for each exposure time. Following scarification seeds were treated as previously described and incubated at 35°C.

Figure 2.3. Pneumatic seed scarifier (Model PSS2000, OEM, Inc.)
Used for mechanical scarification of Eragrostis eriopoda and Fimbristylis dichotoma seeds.

2.3.8 Statistical analysis

The data was recorded onto worksheets or directly entered into a data logger for loading into Microsoft Excel (version 14). Data were analysed using regression analysis and Kruskal Wallis to check the association among the variables and their impact on the dependent variable using GenStat version 18 (Copyright 2016, VSN International Ltd) no data transformation was needed. Factors included were incubation temperature, germination stimulants, time period (afterripening and wet/dry cycling only), grade and exposure duration of abrasive paper and acid concentration, and duration of exposure. Significant differences between treatments were detected by using Fisher's LSD as the multiple comparison tests. Time to 50% of the maximum germination was also calculated using GERMINATOR software (version 2010, Joosen *et al*. 2010).

2.4 Results

2.4.1 Seed quality

Three different embryo types were identified across the seven species assessed, namely; lateral, capitate, and spatulate (Table 2.2). One thousand seed weight ranged between 0.15 to 1.64 g. The E:S ratio ranged from 0.33 g (*Fimbristylis dichotoma*) to 0.66 g (*Goodenia stobbsiana*). Seeds from all seven species had high fill (X-ray) (>86%) and viability (tetrazolium test) (>75%) (Figure 2.3, Table 2.2).

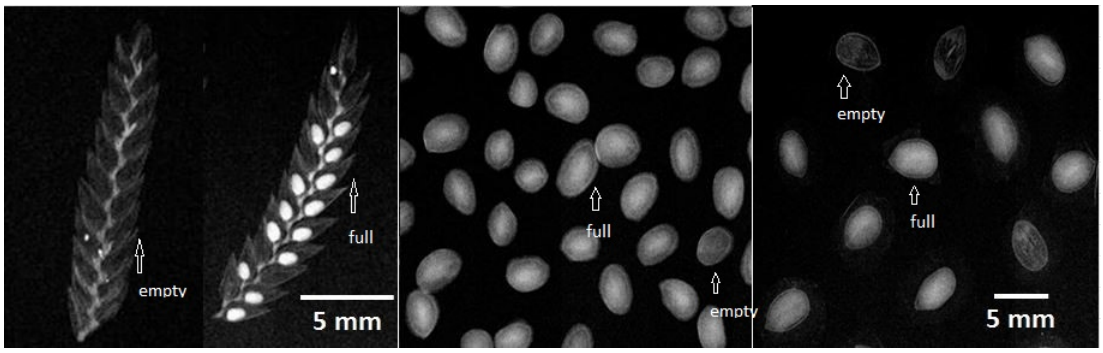

Figure 2.4. X-Ray images that illustrate: left) Empty and full florets of Eragrostis eriopoda; middle) Goodenia cusackiana seeds; and right) Goodenia armitiana seeds.

Table 2.2. Fruit, seed and embryo characteristics of the seven study species.

Family	Species	Life form	Plant form	Dispersal unit	1000-Seed weight (g)	Embryo type	E:S ratio	Seed fill (%)	Viability (%)
Poaceae	*Cymbopogon obtectus*	P	G	Seed	0.65	L	0.45	100	96.0±3.3
Poaceae	*Eriachne mucronata.*	P	G	Seed	0.86	L	0.34	86	86.0±4.4
Poaceae	*Eragrostis eriopoda*	P	G	Seed	0.15	L	0.36	88	75.0±3.3
Cyperaceae	*Fimbristylis dichotoma*	P	G	Seed	0.18	C	0.33	96	85.0±5.8
Goodeniaceae	*Goodenia stobbsiana*	P	S	Seed	0.75	Sp	0.66	95	NA
Goodeniaceae	*Goodenia armitiana*	P	H	Seed	1.64	Sp	0.63	99	NA
Goodeniaceae	*Goodenia cusackiana*	P	H	Seed	1.40	Sp	0.62	95	NA

Life form: A= Annual, P= Perennial; **Plant form:** H= Herb, G= Grass, S= Shrub; **Embryo type:** L= Lateral, C= capitate, Sp= Spatulate; **E:S ratio**= Embryo to Seed length ratio, **NA**= Not Applicable.

2.4.2 Seed coat permeability to water

Seeds of all seven species readily imbibed water when placed onto a moistened substrate (Figure 2.5). Increase in seeds mass due to water uptake ranged from 42.3% (*Fimbristylis dichotoma*) to 632.6% (*Goodenia armitiana*) over different time periods (24 h – 72 h)

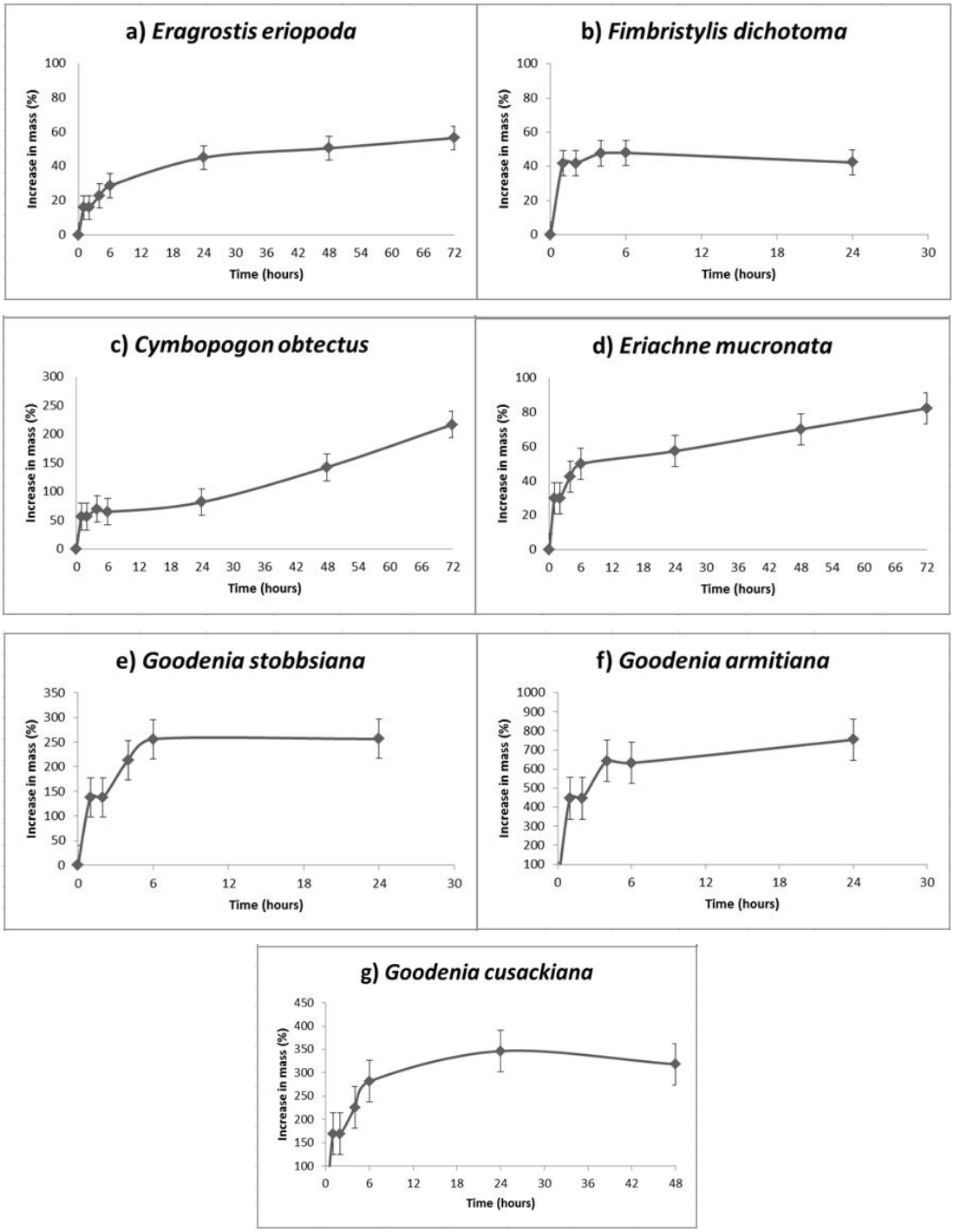

Figure 2.3. Percentage (± s.e.) increase in mass (i.e. imbibition) of seeds from the seven study species.
Seeds under standard laboratory conditions (~23°C). Note the differing x and Y-axis scales between species.

2.4.3 Germination test

2.4.3.1 Initial germination assessment

No germination was observed for the seeds of *Eragrostis eriopoda* when incubated at 20, 25, 30, 35, 20/35, 50 and 25/50°C. Some germination was however observed at 40, 25/40 and 25/45°C, though in all cases it did not exceed 5 (approx. %) and seeds germinated both in the presence of water or 0.67 µM KAR_1 (Figure 2.6.a).

Likewise, no germination was observed for *Fimbristylis dichotoma* seeds when incubated at 20, 25, 30, 35, 50 and 25/50°C though some germination was observed at 40 and 25/40°C, with higher germination obtained in the KAR_1 exposed seeds incubated at 25/40°C (Figure 2.6.b).

Germination of *Cymbopogon obtectus* seeds was high (>70%) across all four temperature regimes though germination appears to have been a little lower at 35°C compared to the three other temperature regimes assessed (Figure 2.6.c). Overall, germination in response to KAR_1 was generally similar to germination in response to water though KAR_1 treated seeds showed lower germination at 35°C compared to seeds exposed to water only. Seeds treated with GA had the lowest germination across all temperatures (Figure 2.6.c) which was significantly lower than either water or KAR_1 treated seeds ($p<0.05$). *Eriachne mucronata* germination was also significantly high (>80%) in all four temperatures 20, 25, 30, 35°C in both water and KAR_1 exposed seeds ($p<0.05$). However, seeds exposed to GA_3 had the lowest germination in all four temperatures regimes which was significantly lower for seeds incubated at 20, 30, or 35°C compared to either water or KAR_1 exposed seeds ($p<0.05$; Figure 2.6.d).

Goodenia stobbsiana germination was significantly higher in seeds incubated on the GA_3 medium in all temperature regimes assessed excluding under the 25°C , compared to seeds exposed to water and KAR_1 media ($p<0.05$). Seed germination on water medium was similar across all temperatures and did not exceed 16%. Germination percentage on KAR_1 medium was significantly higher at 25°C reaching 47% and was similar to the germination observed at the same temperature for seeds incubated on the GA_3 medium ($p<0.05$; Figure 2.6.e).

Highest germination for *Goodenia armitiana* seeds was observed to occur at either 25°C or 20/35°C with germination at the two other temperature regimes much lower (Figure 2.6.f). There were no clear differences in most cases between the germination

stimulants assessed with seeds incubated on water, GA_3 or KAR_1 germinating to similar levels ($p > 0.05$) though seeds exposed to GA_3 and incubated at 25/45°C did display the lowest germination overall (Figure 2.6.f).

As observed for *G. armitiana*, the highest germination for *G. cusackiana* seeds was observed at either 25°C or 20/35°C, with germination at the two other temperature regimes much lower (Figure 2.6.g). Seeds exposed to KAR_1 germinated to a much higher level in three out of the four temperatures assessed and proved overall to be more effective at promoting germination than either water or GA_3 (Figure 2.6.g). In comparison, germination in response to GA_3 exposure was only significantly improved at 25°C when compared to the water treated seeds though this was still marginally lower than the germination observed for the KAR_1 treated seeds incubated at the same temperature ($p<0.05$).

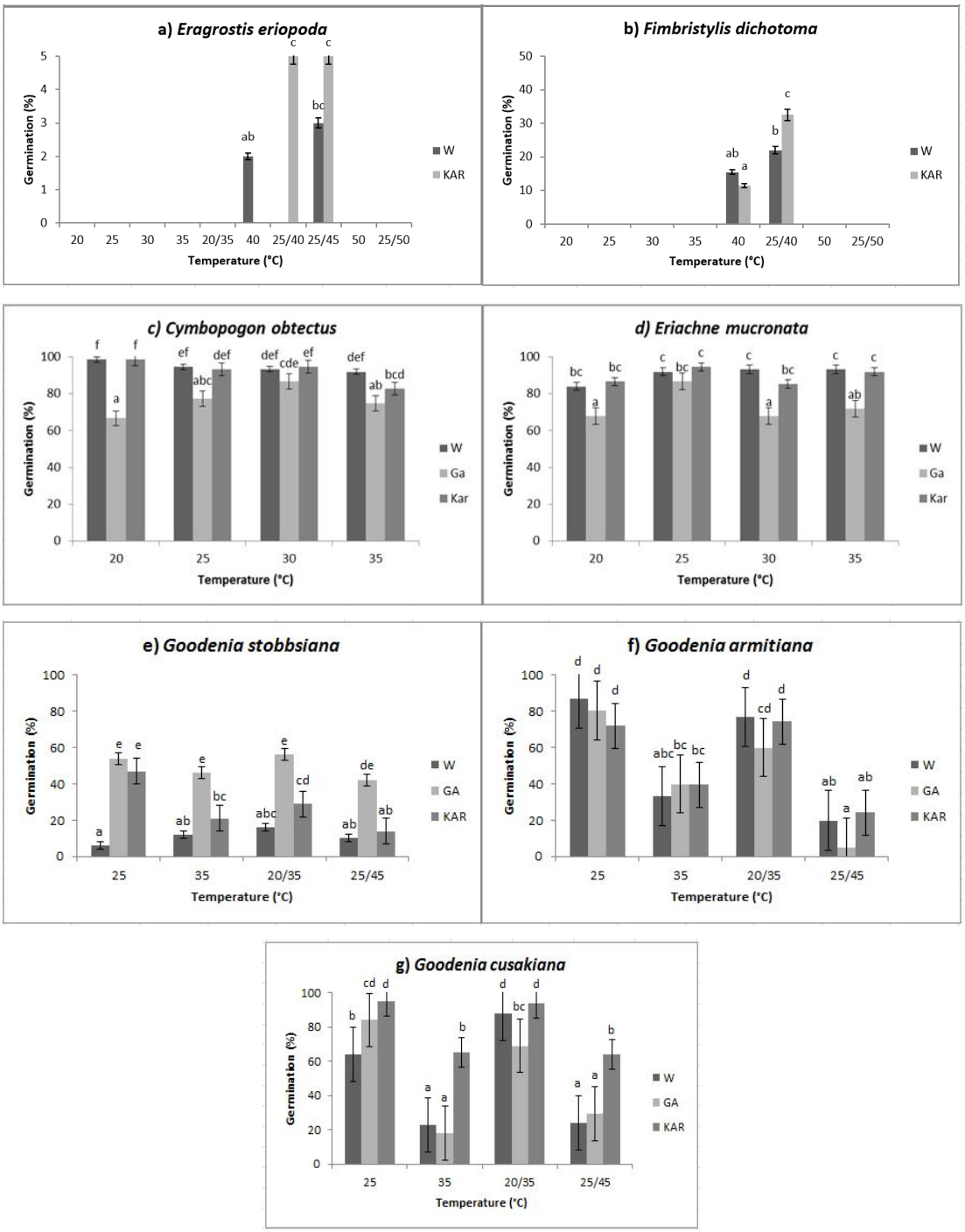

Figure 2.4. Percentage of germinated seeds (± s.e.) from the seven study species *under different incubation temperatures and germination stimulants. (NOTE: for Eragrostis eriopoda and Fimbristylis dichotoma germination was also assessed in the presence of GA_3 at four temperatures (20, 25, 30, and 35°C) which resulted in 0% germination (data not shown)). Note the differing y-axes scales between species (w=water, GA= Gibberellic acid, KAR=Karrikinolide).*

2.4.3.2 Germination in presence of florets

Of the two species used in this study, *Cymbopogon obtectus* germination did not differ significantly in most of the media and temperature combinations assessed ($p>0.05$; Figure 2.7.a,b). The presence or absence of florets surrounding the seeds was also found to have no substantial impact on germination (Figure 2.7.b). However a modest reduction (~10 %) in germination was noted with the presence of florets (Figure 2.7.a,b).

In contrast, the germination of *Eriachne mucronata* seeds without florets differed significantly compared to when florets were present recording much higher germination (~2 fold improvement) in all media and temperature combinations ($p<0.05$). Germination in *Eriachne mucronata* without florets in all media and temperature was similar and reached 92% - 98% (Figure 2.7.d); however, with florets it was much lower with seeds exposed to the GA_3 treatments in particular performing very poorly compared to either water or the 1 µM KAR_1 treatments (Figure 2.7.c).

Eragrostis eriopoda did not germinate under any temperature and treatment combinations (data not shown).

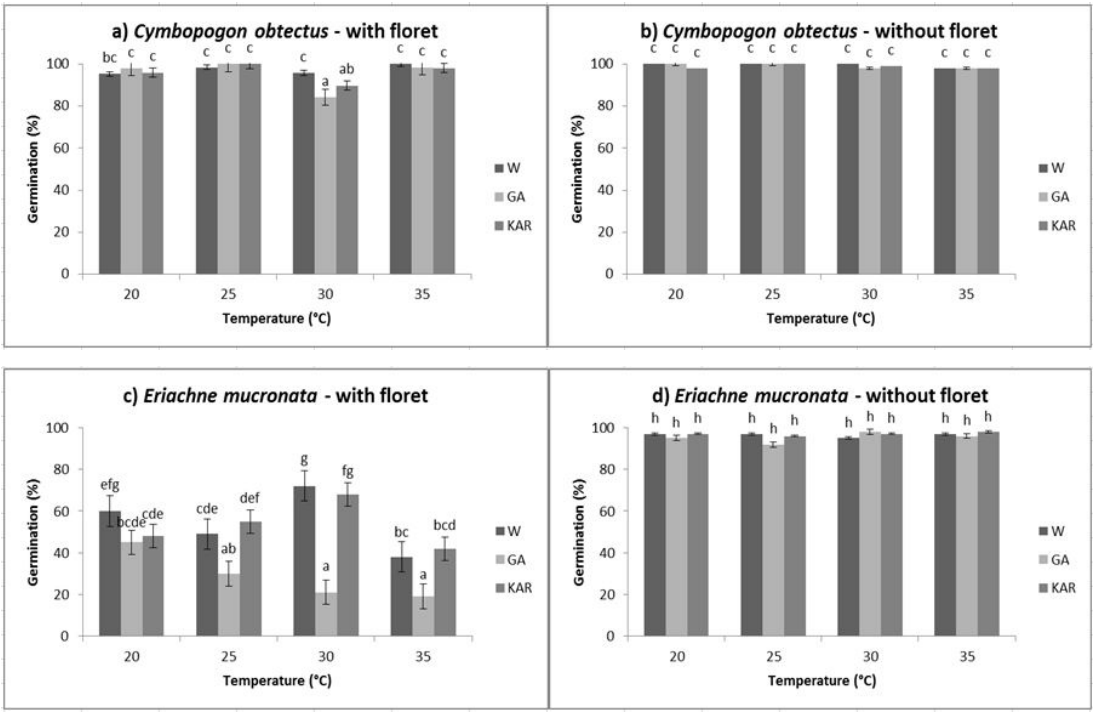

Figure 2.5. Percentage of germinated seeds (mean ± s.e.) of two species when plated with or without florets: a) & b) *Cymbopogon obtectus;* c) & d) *Eriachne mucronata* under different temperature and treatments. (*Eragrostis eriopoda did not germinate and so the data is not shown).*

2.4.4 Effect of afterripening and wet/dry cycling treatments on seed germination

2.4.4.1 Poaceae and Cyperaceae species

Prior to afterripening negligible germination was observed for seeds of *E. eriopoda* at either the 30°C and 20/35°C incubation temperature regimes (Figure 2.8). However, ~2% germination was recorded from the 25/45°C temperature regime. There was no effect of the KAR_1 treatment on seed germination with similar germination patterns observed between water and KAR_1 treated seeds ($p>0.5$; Figure 2.8). However, following afterripening for 6 months germination was improved. For after ripened seeds, germination was highest ($p<0.05$) for seeds maintained at 50°C compared to those kept at either 40°C or 60°C. This was further improved when combined (for germination assessment) with incubation of seeds at 25/45°C resulting in germination >15%. Some germination (up to 10%) was also observed for seeds incubated at 20/35°C as well (Figure 2.8).

No germination was observed for *Eragrostis eriopoda* and *Fimbristylis dichotoma* seeds or florets in response to either H_2O or KAR_1 treatment following afterripening for up to 18 months when incubated at 25°C (data not shown). However, afterripening *Fimbristylis dichotoma* under the same conditions then incubation at 40°C decreased seed germination compared to control treatments exposed to the same incubation conditions prior to afterripening (Figure 2.8.c).

Exposure of seeds from *Eragrostis eriopoda* and *Fimbristylis dichotoma* to wet/dry for either seeds or florets and treatments for up to 18 months then incubation at 25°C resulted in no germination (data not shown). However, *Fimbristylis dichotoma* treated in a similar way then incubated in water at 40°C showed higher germination (>50%) compared to the control treatment, which had only 30% germination ($p<0.05$; Figure 2.8.c).

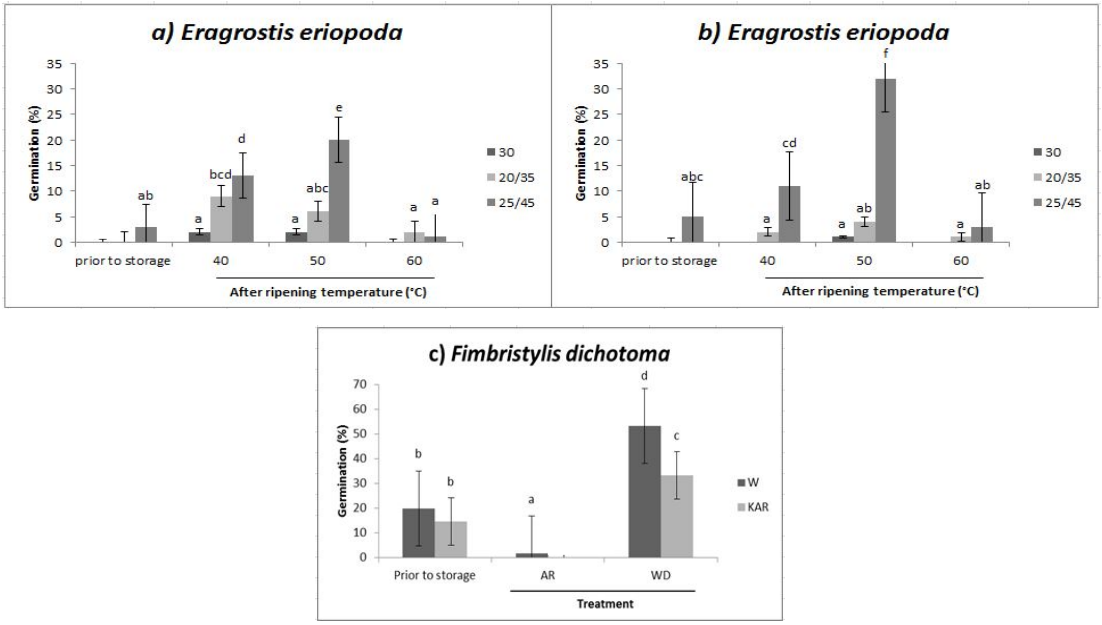

Figure 2.6. top) Percentage of germinated seeds (mean ± s.e.) of *Eragrostis eriopoda* in a) H_2O and b) KAR_1 prior to storage or following afterripening for 26 weeks at either 40°C, 50°C, and 60°C) percentage of germinated seeds (mean ± s.e.) of *Fimbristylis dichotoma* in H_2O and KAR_1 prior to storage or following afterripening (AR) and wet/dry (WD) for 18 months at 30°C and 50% RH and incubation at 40°C.

2.4.4.2 *Goodenia* species

2.4.4.2.1 *Goodenia stobbsiana*

Prior to afterripening or the application of wet/dry treatments, germination across the four temperature regimes assessed was generally quite similar, ranging from 10 - 20 % in response to water, up to 30 – 50 % in response to 1 µM KAR_1 (Figure 2.9). The highest germination overall (~50) prior to the assessment of dormancy breaking treatments was observed for seeds exposed to KAR_1 and incubated at 25°C.

The application of wet/dry treatments did not improve germination over time with most treatment combinations showing much lower germination (<5% germination) compared to germination observed at the beginning of the experiment. Likewise, there were no differences ($p>0.05$) in germination between seeds either treated with water or exposed to KAR_1 after wet/dry, regardless of both the time of assessment (i.e. 1 to 8 months) or the incubation regime used (Figure 2.9).

In comparison, dry afterripening improved germination in many treatment combinations and was noticeable after only 1 month of afterripening when seeds were exposed to water only and incubated at 25°C, 35°C and 25/45°C (Figure 2.9). However under temperature regimes of 35°C and 25/45°C it started to drop in the remaining longer

afterripening treatments. KAR_1 treatment also improved germination over time though a minimum of 3 months afterripening was needed to improve germination in this case. Highest germination (~63%) following afterripening was observed for those seeds exposed to KAR_1 after 8 months storage then incubated at 25°C, though the germination observed at 20/35°C after the same sequence of treatments (i.e. 8 months afterripening and KAR_1 exposure) was also quite similar (~58%). In comparison, the germination observed in response to the other two temperature regimes assessed i.e. 35°C and 25/45°C was much lower, never rising above 30 to 40% regardless of how long seeds were after ripened (Figure 2.9).

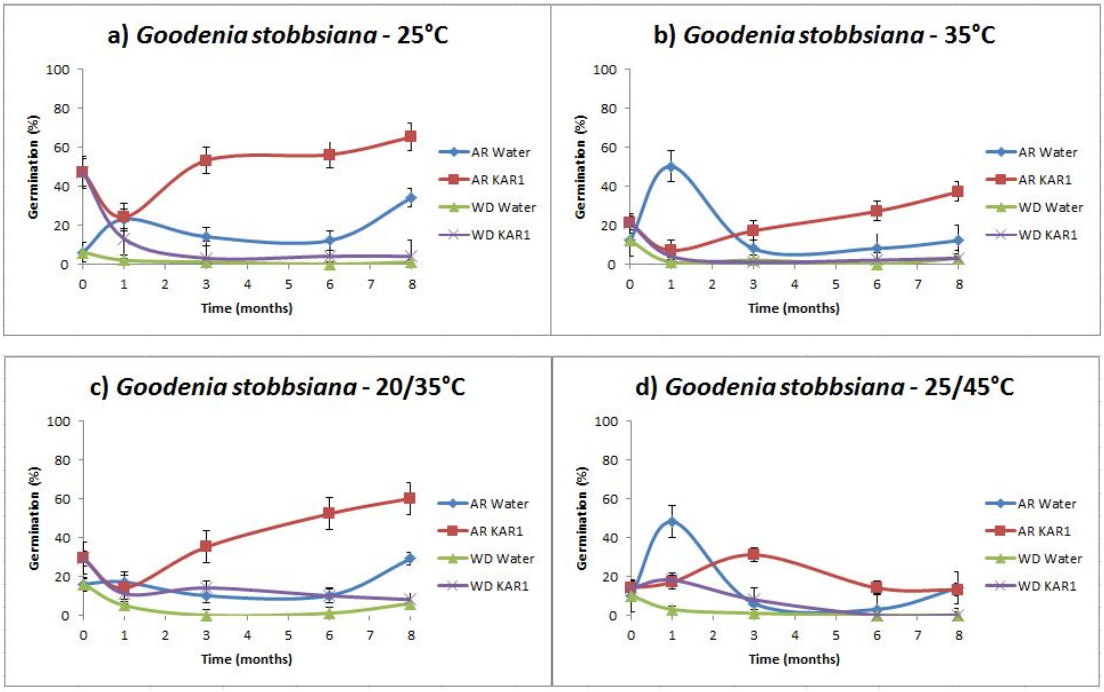

Figure 2.7. Percentage of germinated seeds (mean ± s.e.) of *Goodenia stobbsiana* after (1,3,6,8 months) of incubation at four different temperature regimes following afterripening or wet/dry conditions (30°C and 50% RH) for up to eight months.

2.4.4.2.2 *Goodenia armitiana*

Prior to afterripening or the application of wet/dry treatments, germination across the four temperature regimes assessed was quite different (Figure 2.10). Germination ranged from ~ 20% in response to either water or 0.67 µM KAR_1 for seeds incubated at 35°C or 25/45°C to higher than 70% for seeds incubated at either 25°C or 20/35°C (Figure 2.10). Apart from a small (~12%) difference in germination between water (~88%) and KAR_1 (76%) treated seeds when incubated at 25°C there was no significant differences between water and KAR_1 seed treatments prior to the assessment of either afterripening or wet/dry regimes ($p>0.05$; Figure 2.10).

Similar to the seeds of *G. stobbsiana*, the application of wet/dry treatments to the seeds of *G. armitiana* did not improve germination over time with most treatment combinations showing lower germination compared to seeds that were exposed to afterripening conditions ($p>0.05$; Figure 2.10). In most cases germination was also lower than the germination ($p>0.05$) observed at the beginning of the experiment prior to the assessment of wet/dry dormancy alleviation treatments. Likewise, there were no differences ($p>0.05$) in germination between seeds either treated with water or exposed to KAR_1 after wet/dry treatments, regardless of either the time of assessment (i.e. 1 to 8 months) or the incubation regime used. However differences occured in different incubation temperatures. The largest difference between the water and the KAR treatment noted was in seeds incubated at 25°C after 8 months of wet/dry treatment where a ~25% difference was observed between water-treated seeds (57% germination) and those that were exposed to 0.67 µM KAR_1 (82% germination) (Figure 2.10).

Due to the high germination (>70%) observed for seeds incubated at either 25°C and 20/35°C prior to the assessment of afterripening treatments, there were only modest improvements in the overall germination for seeds incubated under the same temperature regimes over different sampling times (Figure 2.10). After 8 months afterripening the highest germination was observed for seeds incubated at 25°C in the presence of either water (>90%) or 1 µM KAR_1 (>90%) which displayed very similar germination to one another. In comparison, seeds incubated in the presence of 0.67 µM KAR_1 at 20/35°C also displayed high germination (>88%) though seeds germinated to <60% in the presence of water showed much lower germination ($p<0.05$) at the same incubation temperature (20/35°C) compared to the KAR_1 treated seeds. This large difference in germination between water and KAR_1 treated seeds was also observed for seeds assessed at earlier sampling times (i.e. 3 and 6 months) under the same germination conditions (i.e. 20/35°C) (Figure 2.10).

Initial germination at the two warmer incubation temperatures (35°C and 25/45°C) was much lower (<30%) so consequently improvements in germination over time for these two incubation temperatures were in many cases more substantial. For example, germination increased from <30% to >55% (KAR_1 treatment) for seeds after ripened for 6 months which was the highest germination observed for seeds incubated at 25/45°C. More significantly, germination for *G. armitiana* seeds rose from ~30% for both water and KAR_1 treated seeds prior to afterripening to >90% after 8 months afterripening

treatment when seeds were incubated at 35°C which was higher than most other treatment combination assessed as part of this experiment (Figure 2.10).

Afterripening of seeds for different amounts of time (1 to 8 months) was also found to consistently improve germination when combined with KAR_1 treatment of after ripened seeds. KAR_1 also improved germination across the various incubation temperatures and depending on the sampling time and incubation temperature regime improved germination from 10 to 70% when compared to seeds exposed to water only (Figure 2.10).

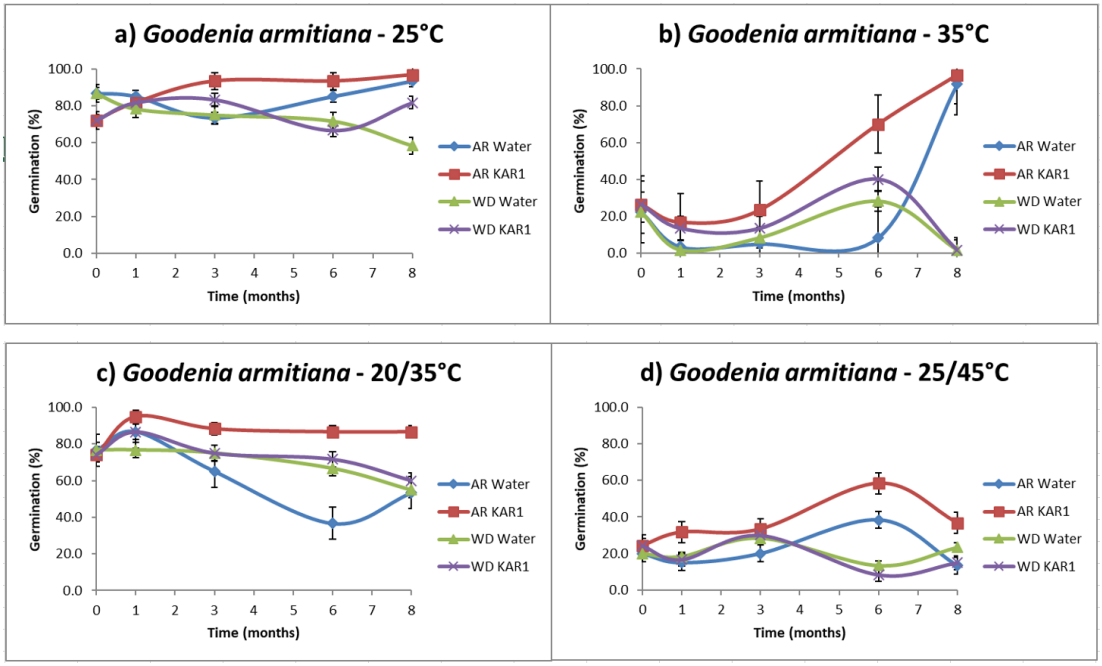

Figure 2.8. Percentage of germinated seeds (Mean ± s.e.) of *Goodenia armitiana* at four different temperature regimes following afterripening or wet/dry conditions (30°C and 50% RH) for up to eight months.

2.4.4.2.3 *Goodenia cusackiana*

Prior to afterripening or the application of wet/dry treatments, initial germination across the four temperature regimes assessed fell into two distinct groups. Seeds incubated at 25°C and 20/35°C germinated to >65% in the presence of either water or KAR_1 with few differences between the treatments. In comparison, those incubated at 35°C and 25/45°C germinated to much lower levels; around 25% for seeds incubated on water only while those exposed to KAR_1 germinated to ~60% (Figure 2.11).

The application of wet/dry treatments to the seeds of *G. cusackiana* did not improve germination over time with all wet/dry treatment combinations showing lower germination compared to seeds that were exposed to both afterripening and KAR_1

conditions (Figure 2.11). In most cases germination was also lower than the germination observed at the beginning of the experiment prior to the assessment of wet/dry dormancy alleviation treatments ($p<0.05$). In terms of interactions between wet/dry treatments and water or KAR_1 incubation, KAR_1 proved to be more effective in most cases increasing germination by up to 40% though for several retrievals times and incubation temperatures (i.e. 6 months wet/dry & 20/35°C incubation and 6 months wet/dry & 25/45°C incubation) the germination observed from the water treatments was higher than the KAR_1 treatments (Figure 2.11).

Due to the high germination (>90%) observed for seeds incubated at either 25°C and 20/35°C prior to the assessment of afterripening treatments there were no meaningful improvements ($p>0.05$) in the overall germination for seeds incubated under the same temperature regimes over different sampling times (Figure 2.11). Germination from seeds exposed to the KAR_1 treatment in particular remained both similar and consistent (>90%) over 8 months afterripening though seeds incubated on water only over the same sampling period showed much more variable germination (Figure 2.11). On most occasions germination from water treatments was less than half (i.e. < 45%) the germination observed from the corresponding KAR_1 treatments (Figure 2.11).

As the initial germination at the two warmer incubation temperatures (35°C and 25/45°C) was much lower (~22% and ~62% for water and KAR_1 treated seeds, respectively) improvements in germination over time for these two incubation temperatures were in several instances more substantial. For example, germination increased from ~22 – 60% to >90% (both water and KAR_1 treatments) for seeds afterripened for 8 months then incubated at 35°C (Figure 2.11). However, for those seeds afterripened then incubated at 25/45°C germination overall declined, dropping from 22 – 60 % prior to afterripening down to only 10 – 40% after 8 months afterripening (Figure 2.11). As with the germination patterns observed with the two cooler temperature regimes seeds incubated on KAR_1 consistently germinated to a higher percentage compared to those incubated in the presence of water only - germinating up to 60% + higher on occasion (i.e. 6 months afterripening then incubation at 35°C). However, the germination differences were usually much smaller than this ($p>0.05$); around 20% between water incubated seeds and KAR_1 incubated seeds when compared at the same sampling time and incubation temperature (Figure 2.11).

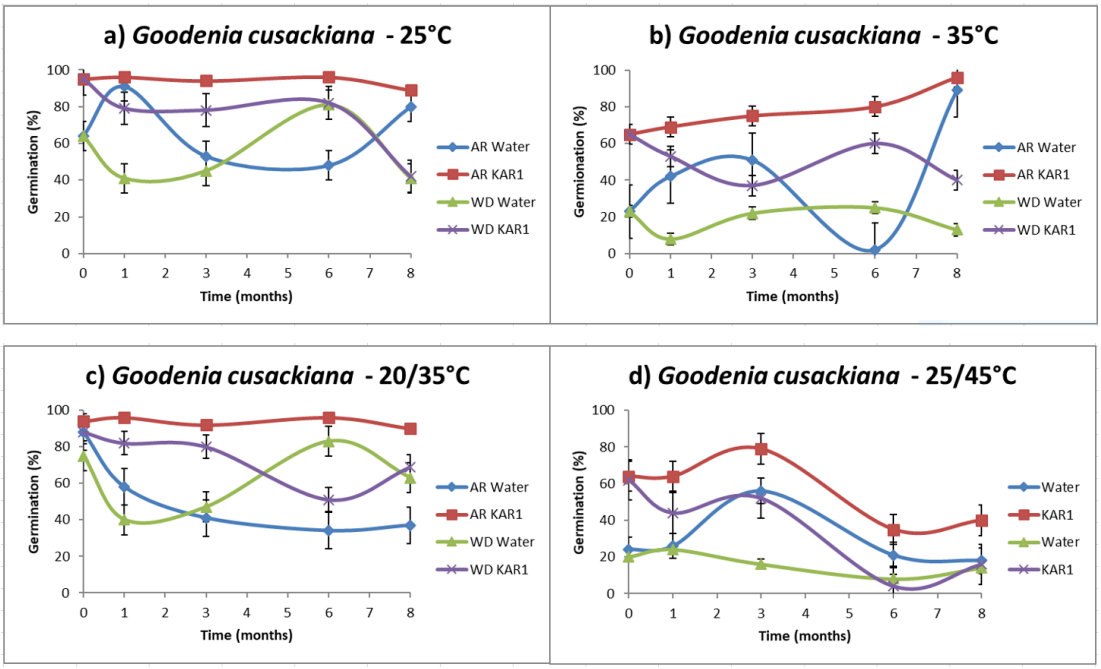

Figure 2.9. Percentage of germinated seeds (Mean ± s.e.) of *Goodenia cusackiana* at four different temperature regimes following afterripening or wet/dry conditions (30°C and 50% RH) for up to eight months.

2.4.5 Effect of scarification on seed germination

2.4.5.1 Acid scarification

Eragrostis eriopoda germination following acid scarification did not exceed 20 % which was achieved following 60 minutes treatment with 50% (v/v) H_2SO_4 then incubation of seeds at 40°C on KAR_1 medium. While low, this result was nevertheless significant compared to most of the other treatments that were assessed ($p<0.05$; Figure 2.12). Also significant was the result obtained following 30 minutes H_2SO_4 treatment then the incubation of seeds on KAR_1 medium at 25/45°C which resulted in ~ 10% germination, which was also similar for seeds incubated on water at 40°C after 60 minutes acid exposure ($p<0.05$; Figure 2.12). In general, the treatment of seeds with H_2SO_4 improved germination compared to non-treated seeds with 60 minutes exposure proving to be more effective than 30 minutes exposure time though this effect was more variable for seeds incubated at different incubation temperatures on KAR_1 medium ($p<0.05$; Figure 2.12).

When assessed at the optimal incubation temperatures (i.e. 40°C, 25/40°C) (Figure 2.6) the germination of *Fimbristylis dichotoma* seeds was also higher following H_2SO_4 treatment ($p<0.05$; Figure 2.12). The effects of acid scarification were however more noticeable for seeds incubated on water compared to those exposed to KAR_1 with 60 minutes proving to be more effective in promoting germination compared to 30 minutes exposure time (Figure

2.12). Highest germination overall (~47%) was achieved following treatment for 60 minutes in H_2SO_4 then incubation of seeds at 25/40°C on KAR_1. Germination >35% was also achieved in several other treatments following the exposure of seeds for 60 minutes to H_2SO_4 though in these cases the seeds were incubated on water at either 20/35°C and 25/40°C rather than on KAR_1 (Figure 2.12).

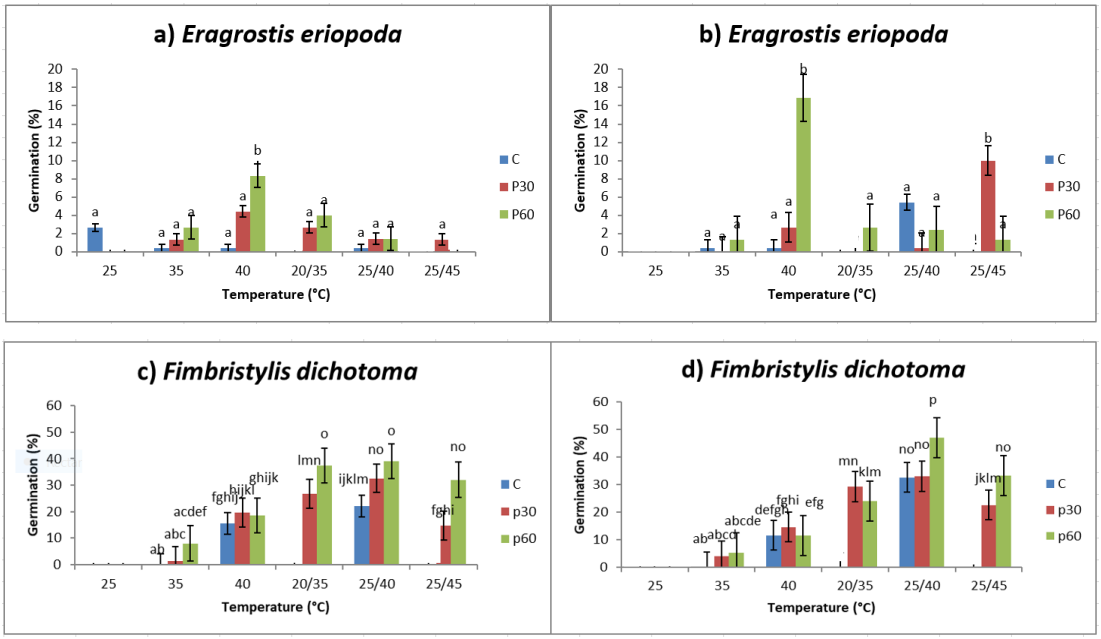

Figure 2.10. Mean germination (% ± s.e.) of *Eragrostis eriopoda* and *Fimbristylis dichotoma* seeds after two acid scarification (50 % (v/v) H_2SO_4) treatments and incubation under different conditions: a and c) H_2O and b and d) 0.67 µM KAR_1. (C: control, P30 abrasive paper 30 mins and P60: abrasive paper 60 mins).

2.4.5.2 Mechanical scarification

Eragrostis eriopoda germination percentage following mechanical scarification was no different ($p>0.05$) for most of the treatment combinations assessed (Table 2.3). Only four out of the 20 scarification treatments resulted in germination, which was generally low (< 1%) rising above 1% in only two treatment combinations (Table 2.3). The highest germination on H_2O agar was obtained using 180 (fine) grade paper for 5 minutes which reached 5.9 ± 0.7%. Germination for the same scarification treatment (i.e. 180 grade paper and 5 minutes treatment time) was further improved using KAR_1 incubation medium reaching 12% (Table 2.3). Likewise, *Fimbristylis dichotoma* germination was generally poor with only nine out of the 20 scarification treatments germinating (Table 2.4). Germination where it occurred was generally quite similar across the scarification treatments ranging from 2 to 9% with no clear improvements observed for seeds incubated in the presence of KAR_1 (Table 2.4).

Table 2.3. Effect of mechanical scarification on percentage of germination (mean ± s.e.) of *Eragrostis eriopoda* seeds incubated on H_2O or KAR_1 media at 35°C temperature.

	Grit number					Grit number				
Duration	**0**	**60**	**80**	**120**	**180**	**0**	**60**	**80**	**120**	**180**
	H_2O agar					**KAR_1 agar**				
0 (control)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
30 sec	0.0	0.0	0.0	**0.9±1.2**	0.0	0.0	0.0	0.0	0.0	0.0
1 min	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2 min	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**1.0±1.2**	0.0	0.0
5 min	0.0	0.0	0.0	0.0	**5.9±0.7**	0.0	0.0	0.0	0.0	**12.0±0.7**
10 min	0.0	**0.9±0.3**	0.0	0.0	0.0	0.0	**0.9±0.3**	0.0	0.0	0.0

Table 2.4. Effect of mechanical scarification on percentage of germination (mean ± s.e.) of *Fimbristylis dichotoma* seeds incubated on H_2O or KAR_1 media at 35°C temperature.

	Grit number					Grit number				
Duration	**0**	**60**	**80**	**120**	**180**	**0**	**60**	**80**	**120**	**180**
	H_2O agar					**KAR_1 agar**				
0 (control)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
30 sec	0.0	0.0	**9.0±2.2**	**8.0±1.9**	0.0	0.0	0.0	**4.0±1.8**	**6.0±1.3**	0.0
1 min	0.0	**5.0±3.5**	**4.0±2.7**	0.0	0.0	0.0	**4.0±3.2**	**6.0±1.3**	0.0	0.0
2 min	0.0	**4.0±3.3**	**3.0±2.2**	0.0	0.0	0.0	**8.0±3.2**	**3.0±1.1**	0.0	0.0
5 min	0.0	**2.0±0.3**	0.0	0.0	**4.0±0.8**	0.0	**5.0±0.5**	0.0	0.0	**7.0±0.7**
10 min	0.0	0.0	0.0	0.0	**6.0±0.7**	0.0	0.0	0.0	0.0	**7.0±0.7**

2.5 Discussion

This study focused on the seed biology of seven perennial species with different plant forms from the Pilbara region of Western Australia that are required for mine site restoration. The seed and embryo characteristics along with the capacity of seeds to imbibe water and germination testing were used to identify the class of dormancy present in each species. All species had seeds with high viability which could absorb water rapidly indicating that they do not have physical dormancy so were either non dormant, or had physiological seed dormancy (Baskin and Baskin, 2004a). Germination of seeds varied among the species with *Eragrostis eriopoda* and *Fimbristylis dichotoma* in particular showing very low initial germination. In both species no germination was observed across a wide range of temperatures (20, 25, 30, 35, 50, 25/50°C) with only a few germinants found at either 40 and 25/40°C. Since both species have a fully developed embryo (lateral and capitate) they

were determined to have physiological seed dormancy as found by Allessio Leck and Schütz (2005), Erickson et al. (2016b) and Commander et al. (2017) in several studies on these and other closely related species. Interestingly, it has also previously been reported that germination of *E. eriopoda* reached ~40% when seeds were incubated at very hot temperatures, i.e. 42°C (Ross, 1976). This verifies our findings that showed *E. eriopoda* need high temperature in order to germinate, however germination did not exceed 40%.

The response to GA_3 was used to differentiate between the three level of physiological dormancy, namely; non deep, intermediate and deep PD (Baskin and Baskin, 2004a). Since there was no response to GA_3 when seeds were incubated at different temperatures this strongly suggests that the seeds of both species have deep physiological dormancy. In contrast, the seeds of *Cymbopogon obtectus* and *Eriachne mucronata* were found to have high germination across all the temperature regimes tested (i.e. 20, 25, 30, and 35°C) which indicates that these seeds are largely non dormant. This result is similar to another study that also characterized *Cymbopogon obtectus* as not dormant (Erickson, 2015; Erickson et al., 2016b).

The seeds of *Goodenia stobbsiana* were also found to have low germination but once treated with GA_3 germination increased significantly ($p<0.05$) across all the temperature regimes investigated which indicates that these have non-deep PD. This finding is similar to other studies that have also classified it as having PD (Erickson et al., 2016b; Commander et al., 2017). In contrast, the seeds of *Goodenia armitiana* were found not to be stimulated by any of the treatments applied though had higher germination at either 25 or 20/35°C, which suggests that the seeds of this species are largely non dormant. Our results contrast with another study where it was found that GA_3 and KAR_1 improved germination of *Goodenia armitiana* and may be due to differences in storage conditions prior to germination assessment or differences between collection locations and years as reported for other species with variable germination (Gorecki et al. 2012; Commander et al., 2017). High germination (>60%) of *Goodenia cusackiana* was found when seeds were incubated at 25 and 20/35°C. While much lower germination was observed from the other two temperatures assessed (35°C and 25/45°C) this was still significantly improved when seeds were exposed to KAR_1 which effectively doubled germination in both cases.

The presence of florets surrounding the seeds *Cymbopogon obtectus* and *Eriachne mucronata* impacted germination in two distinctive ways, though in both cases seeds under some conditions still germinated to a high percentage suggesting that the seeds of both species are largely non dormant. The presence of florets did not affect the germination of

Cymbopogon obtectus seeds which germinated to >80% across all the treatments assessed. However, the seeds of *Eriachne mucronata* generally showed lower germination when seeds were retained within the floret which may indicate that the presence of this structure was restricting germination in some capacity though there were several treatments (30°C on water agar or 30°C on KAR_1) where germination >60% was still observed. This restriction of germination due to the presence of surrounding florets is similar to the results reported in other studies where the removal of seeds from covering structures in several *Triodia* species (Poaceae) significantly improved germination from around 0 - 30% to higher than 70% in some cases (Erickson et al., 2016).

Species in arid lands mainly exhibit physiological dormancy which is the single biggest class of seed dormancy generally found in these environments (Baskin, and Baskin, 1998). It was concluded from seed assessment and germination tests that both *Eragrostis eriopoda* and *Fimbristylis dichotoma* seeds have deep physiological dormancy due to their low germination, and lack of response to GA_3. Afterripening the seeds of *Eragrostis eriopoda* at 50°C for 6 months then incubating these at 25/45°C was found to result in higher germination compared to the other treatments assessed which generally did not result in any germination whatsoever, including the wet/dry cycling treatment. In comparison, the germination of *Fimbristylis dichotoma* was significantly improved by the wet/dry cycling treatment after it was applied for 18 months, although to see its full effects seeds had to be incubated at 40°C with the lower incubation temperature (i.e. 25°C) proving ineffective for promoting germination even when seeds should be somewhat non dormant. The requirement for high temperatures for promoting germination of *E. eriopoda* and *F. dichotoma* seeds may be explained by the extreme temperatures in the Pilbara that occur during the summer months (i.e. daily average maximum exceeding ~35°C) when rainfall is more likely following cyclonic events (Ross, 1976). In addition, the seeds of both species are also quite small relative to other species (Moles et al. 2005) and need to be within the top 15 mm of the soil surface (Bond et al. 1999) in the region where the soil seed bank will be the warmest during summer.

The application of the wet/dry treatment to overcome dormancy did not affect the germination of *Goodenia stobbsiana* seeds under any of the incubation temperatures that were assessed (i.e. 25, 35, 20/35, 25/45°C). In contrast however, dry afterripening at 30°C was found to be effective in overcoming dormancy with germination increasing after 8 months when seeds were incubated at 25 and 20/35°C in the presence of KAR_1. It was noticed that afterripening initially increased germination after only 1 month when seeds

were incubated in water at 35 and 25/45°C, however germination decreased significantly after 8 months afterripening when seeds were retested at the same two temperatures. The reason/s for which are unknown at present though in another study on *Dioscorea hastiflolia*, *Anthocercis littorea* and *Zygophyllum fruticulosum* viability declined quite rapidly when seeds were afterripened at 45°C and 50% RH for 3-6 months (Commander et al., 2009).

Afterripening *Goodenia armitiana* seeds increased germination across a range of temperatures and this effect was enhanced when combined with incubating seeds on KAR_1. In addition to afterripening proving to be beneficial under some incubation conditions, there was high initial germination at 25 and 20/35°C as well and for these temperatures there was only a slight increase in germination as seeds were afterripened suggesting that these temperatures are most suitable for *Goodenia armitiana* to germinate. In comparison, incubating seeds at 35°C initially resulted in low germination though afterripening seeds for a period of time improved germination at this temperature, which was noticeable in both the water and KAR_1 treated seeds. For afterripened seeds incubated at 25/45°C germination initially increased up to six months but then decreased for unknown reasons after 8 months. The wet/dry treatment for this species was not useful in overcoming dormancy and appears to have significantly decreased germination compared to the germination without treatments and may be due to the effect of wet/dry treatment on seed vigour.

The initial germination observed in *Goodenia cusackiana* seeds was also high at 25 and 20/35°C though much lower at 35 and 25/45°C but increased over time in response to the afterripening treatment. This result suggests that the seeds of *Goodenia cusackiana* are largely non dormant. Across all temperatures the positive effect of KAR_1 on germination was noted. As with *G. armitiana* the wet/dry treatment was not useful for improving germination and in fact decreased germination. In summary, all three *Goodenia* species responded poorly to the wet/dry treatments which decreased germination across most incubation temperatures, while afterripening in comparison was found to be useful in promoting germination especially when combined with exposing seeds to KAR_1 after the dry afterripening treatment

For acid scarification it was found that sulfuric acid had a slight positive effect on the germination of *Eragrostis eriopoda* and perhaps *Fimbristylis dichotoma* seeds which suggests that the seed coat in both species may be restricting germination and may be overcome by weakening the outer covering layers in the seed. Mechanical scarification

was not as effective in improving germination and may be due to the small size of the seeds which may not have been scarified properly, thus it may be concluded that this method is not useful for very small seeds at present and requires further development.

In conclusion based on the seven species assessed in this study, the germination of arid zone species differed across species in regards to the temperatures required for promoting germination. It was noted that for *E. eriopoda* and *F. dichotoma* high germination temperatures (i.e. ~40°C) were more effective, while for the three *Goodenia* species lower temperatures (~25°C) were more effective. Afterripening was found to improve germination (under some conditions) in most of the species selected while wet/dry treatment was only effective in enhancing germination in *F. dichotoma* seeds. Depending on the species and the other treatments being assessed both GA_3 and KAR_1 improved germination under some conditions though neither proved to be effective across all seven species. While acid scarification was only moderately successful in improving germination, it indicated that the seed coat may have some restrictive effect on germination in the two species tested (i.e. *E. eriopoda* and *F. dichotoma*). These finding have advanced the understanding of seed germination in several plants species endemic to the semi-arid tropical Pilbara region. It highlights the complex dormancy patterns in many species that are required to improve mine-site restoration following land disturbance and clearing. In order to be able to have a full understanding of the germination of the species examined it would valuable to use different temperature regimes not used in this study. For example, a lower temperature that simulates the winter season <20ºC or other combination of temperatures may be necessary.

Chapter 3 Enhancement of seed germination through hydropriming and response to water stress

3.1 Abstract

Poor seedling establishment limits our capacity to restore diverse plant communities and is a problem where plants have developed mechanisms such as seed dormancy to survive in arid regions with irregular rainfall. Priming seeds is often used as a method to enhance and synchronize seed germination, where seeds are pre-soaked in a priming solution (i.e. water) for a predetermined amount of time then redried. Seed priming is currently being used for various plant species for mine-site restoration in Western Australia, though there are still many species that require further research to improve germination and hence be successful in restoration programs. . The purpose of this study was to enhance seed germination of two endemic plant species from the semi-arid Pilbara region in Western Australia that exhibit seed dormancy to enable them to be used in arid land restoration. The seed priming experiment investigated germination speed, germination percentage and the resistance of seeds to water stress. Seeds of *Cymbopogon obtectus* and *Eriachne mucronata* were primed in aerated water for 3 h, 6 h, and 9 h at room temperature (~23°C) then after drying seeds were plated in a range of different water potentials (0, -0.25, -0.5, -1.0 MPa) at 23°C then incubated at 30°C. It was found that combinations of priming treatments significantly increased germination percentage in *Cymbopogon obtectus* under water stress conditions (-1.0 MPa) with the highest percentage germination acquired by 6 h priming compared to the control treatment. All priming treatments significantly enhanced the rate of germination under severe water stress (-1 MPa) although a priming duration of 6 h proved to be more effective for *Cymbopogon obtectus,* whereas priming seeds of *Eriachne mucronata* for 9 h proved to be the most effective treatment in reducing the time to 50% germination when exposed to severe water stress (-1 MPa) compared to the other treatments assessed. It was found that seeds of *Eriachne mucronata* have high germination under severe water stress. Priming had a positive effect on germination parameters such as total germination and germination speed (i.e. time to 50% germination) for seeds under certain water stress. This study assists in better understanding how to improve germination of native species from the arid Pilbara which will improve the success of future revegetation programs.

3.2 Introduction

Revegetation in arid lands is challenged by environmental extremes and in many geographical areas by ongoing human disturbance (Bainbridge, 2007; Hutchinson and Herrmann, 2008). Rainfall in arid lands by definition is limited (on average <300 mm annually), differing each year and varying from winter to summer (Batanouny, 2001). Plants in arid lands possess different adaptations to survive under dry conditions which can include physiological adaptations to control water loss such as stomatal control and foliar water uptake (Basu et al., 2016). However, arid species may possess other adaptations such as avoiding drought, such as winter annuals or where vegetation appears only during the short rainy season. Adaptations may also involve complex germination mechanisms, such as seed dormancy that retain viable seeds in the soil; often for many years (Hawks, 2000).

Moisture and temperature are considered as two of the most important factors in controlling germination in species (Dürr, et al., 2015; Gurvich et al., 2017; Flores et al., 2017). Seedling emergence is a critical stage in the life cycle of native plants in arid environments as moisture has a critical role in determining the distribution pattern of species (Mustart and Cowling, 1993; Schütz, et al., 2002). For example, In a study that examined the impact of low moisture availability on seedling emergence of native cool season species in the arid rangeland of Iran, it was found that soil water content less than -0.6 MPa led to a decrease in the emergence rate, i.e. control and time to 50% germination (t50) while reducing field capacity (FC) by 75% (i.e. -1.5 MPa) lead to zero emergence across all the species assessed (Gazanchian and Malboobi, 2006).

Seed priming is a form of seed treatment used in agriculture and horticulture prior to sowing in which the seeds are pre-soaked in a priming solution (i.e. water) for a predetermined amount of time then redried. Seed priming attempts to improve seed performance in terms of germination rate, uniformity and resistance to stress in specific environments such as arid lands through partially hydrating seeds to trigger initial metabolic processes to a stage before the onset of radicle emergence (Figure 3.2). The capacity of water permeable seeds to absorb water (imbibition) (phase 1) is related to the water potential between the seed and external medium (i.e. soil) as water will diffuse from high water potential (i.e. moist soil) to low water potential (i.e. desiccated seed) thus hydrating the seed. The water potential of a seed cell (Ψ_{Cell}) consists of osmotic water potential ($\Psi_{s)}$), matric potential ($\Psi_{m)}$ and pressure potential (Ψ_p) as in Equation 2.

Equation 2. The water potential of a seed cell adapted from (Kramer, 1995).

$$\Psi_{Cell} = \Psi_s + \Psi_m + \Psi_p$$

- Osmotic water potential ($\Psi_{s)}$ defined through the concentration of dissolved solutes.
- Matric potential ($\Psi_{m)}$ defined by the hydration of matrices such as proteins and cell walls which can prevent water from entering the cell.
- Pressure potential (Ψ_p) is caused by the flow of water into the cell which results in internal pressure.

– **(2)**

Both Ψ_s, Ψ_m have a negative value while Ψ_p has a positive value. The external medium will also have water potential ($\Psi_{external}$); however, the water potential in soil is mainly determined by its Ψ_m unless in highly saline soil then Ψ_s will have relative importance. Soils have water potentials less than zero ($\Psi_{soil}<0$) due to the soil surface area and colloid action of soil particles, while pure water will have $\Psi_{water} = 0$. The water intake will stop when $\Psi_{external} = \Psi_{cell}$ then the second phase of the imbibition/germination process will commence with metabolic activation. However, in seeds undergoing a priming treatment these will be dehydrated at this point, thus $\Psi_{external}$ is reduced to ensure that the seed will not reach the last phase which is the onset of radicle emergence and germination by which time seeds have become desiccation sensitive (Bewley and Black, 1978; Bradford, 1986; Woodstock, 1988).

The curves illustrated in Figure 3.2 highlight the differences in imbibition and germination between un-primed and primed seeds. In both curves the first phase starts with the passive uptake of water when seeds are exposed to moisture, then in the second phase less water uptake occurs but this marks the beginning of seed metabolic activity. However, in primed seeds instead of reaching the third phase which is when cell division and radicle elongation commences, the process is prematurely halted as the seeds are removed from the water and redried and stored for sowing at a later stage (adapted from Lutts et al., 2016).

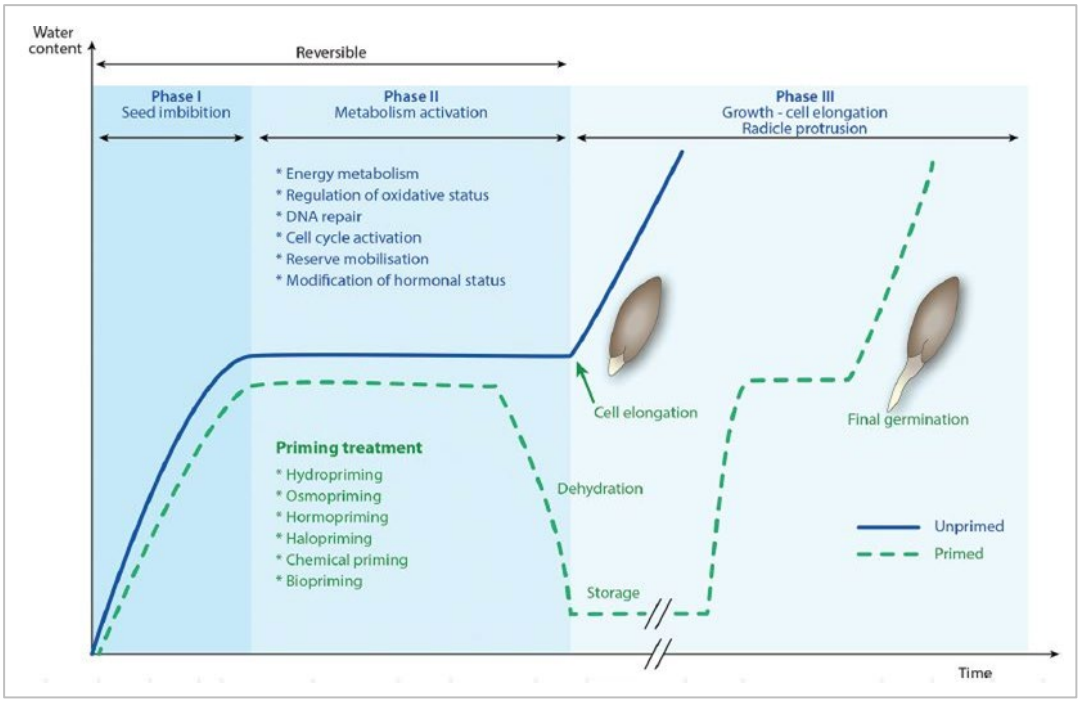

Figure 3.1. Seed hydration curves and germination phases.
In seeds without priming and primed seeds (adapted from Lutts et al., 2016).

Studies have found that priming is useful in several different aspects to increase germination and enhance the emergence rate (Hardegree, 1994; Hardegree and Van Vactor, 2000; Hardegree et al., 2002; Ghassemi-Golezani et al., 2008; Tavili et al., 2010; Qadir et al., 2011; Nouman et al., 2012; Jaghargh et al., 2013; Ahammad et al., 2014), uniformity (Samfield et al., 1991; Farooq et al., 2008), and stress tolerance (Mahmoudi et al., 2012; Theerakulpisut et al., 2017) as well as broadening the temperature window over which seeds will germinate and emerge and improve the capacity of seeds to germinate under low moisture conditions (Schwember and Bradford, 2005; Fallah et al., 2018).

One of the oldest methods of priming is hydropriming which consists of soaking seeds in water under specific temperatures with or without the presence of aeration for up to several days (Paparella et al., 2015). Hydropriming is the simplest priming method as it does not require any chemicals which makes it cost efficient and environmentally friendly; however, one of the biggest disadvantages of hydropriming is uncontrolled water uptake as it depends on the seed tissue permeability to water which can cause imbibition damage if the water uptake rate is too rapid or too long (McDonald, 2000; Lutts et al., 2016). Nevertheless, when effectively applied, studies indicate that hydropriming can enhance seedling emergence, germination rate and improve seedling vigor across a broad range of species. For example, in a study on rangeland grasses, it was found that hydropriming in

distilled water for 24 hours prior to drying was significant in improving emergence percentage and mean emergence time of *Cenchrus ciliaris* when these seeds were sown at a later time (Nouman et al., 2012). In another study on different cotton cultivars hydropriming at 25°C increased germination from 6% to 54% (Bolek and Cokkizgin, 2013). Similarly, hydropriming significantly shortened the emergence time for rice (*Oryza sativa*) when sown in soils with different moisture content and improved seedling vigor as well (Matsushima and Sakagami, 2013).

Osmopriming is another priming method commonly employed to enhance germination attributes that consists of exposing seeds to precisely controlled low water potentials using osmoticums such as Polyethylene Glycol, KNO_3, mannitol, KH_2PO_4, KCl, $CaCl_2$, and $CaSO_4$ (McDonald, 2000). The advantage of osmopriming is to reduce the rate of water intake during imbibition as well as to hold seeds at specific moisture contents below the point where they will germinate (Paparella et al., 2015). Osmopriming can also reduce reactive oxygen species (ROS) - mediated oxidative injury, such as damage to cellular membranes caused by the production of free radicals in fully hydrated seeds (Woodstock, 1988; Varierl et al., 2010; Paparella et al., 2015). One of the main potential disadvantages of osmopriming is toxic side effects due to the osmoticums used with some species unusually sensitive to these compounds (McDonald, 2000). In a study on two f grasses (*Bromus tomentellus* and *Bromus inermis*) both hydropriming and osmopriming treatments were found to significantly improve germination vigour (Tavili et al., 2010). Similarly, osmopriming with a KNO_3 solution was found to enhance both seed germination and seedling vigor in *Cenchrus ciliaris,* while osmopriming with a solution of $CaSO_4$ also resulted in higher germination in *Cenchrus setigerus* and *Panicum antidotale* (Qadir et al., 2011). Priming with a urea (CH_4N_2O) solution positively influenced the germination index, vigor index and final germination percentage in corn (*Zea mays* L.) seeds and highlights the range of solutions that can be successfully used (Dezfuli et al., 2008).

Solid matrix priming is considered as a substitute to osmopriming as it is less expensive and more environmentally-friendly as it does not require the disposal of potentially toxic solutions and chemicals (Olszewski et.al, 2012; Paparella et.al, 2015). This technique is conducted by mixing seeds with solid organic or non-organic material such as charcoal or vermiculite that has been previously moistened and can precisely regulate the rate of imbibition and moisture content inside the seeds (Paparella et al.,

2015). The effect of a six-day treatment of solid matrix priming using Carri-All (clay) on *Pinus taeda* seeds improved the overall germination performance (Wu et al., 2001).

Seeds primed with beneficial microorganisms or bioactive molecules such as rhizobacteria is termed as biopriming (Mahmood et al. 2016). Another type of priming is chemopriming which is used to prevent microbial infection through the use of disinfectants, agrichemicals or other natural substances with abiotic properties (Paparella et al., 2015). Priming *Medicago sativa* L. seeds with brassinolide (a plant hormone) solution under salt stress (13.6 dS/m NaCl solution) improved later germination and seedling growth (Zhang et al., 2007).

Seed priming is considered a new and novel technology in the context of wild species despite its use long before the scientific basis of its beneficial effects were understood (McDonald, 2000; Paparella et al., 2015; Lutts et al., 2016). Several factors may influence the effectiveness of seed priming, for example, in a study on *Zea mays* L., Dezfuli et al. (2008) found that hydropriming improved both germination and the time to 50% germination when compared to osmopriming with either a urea solution or PEG 6000 solution, which were both ineffective (Dezfuli et al., 2008), Similarly, in another study on onion (*Allium cepa*) hydropriming was more effective in improving the speed of germination when compared with osmopriming using aerated PEG 8000 solution (Caseiro et al., 2004).

Different environmental factors during the priming process may affect the success of priming, such as oxygen availability and incubation temperature (McDonald, 2000). For example, it was found that solid-matrix priming in moist vermiculite at three different temperatures (10°C, 15°C, and 20°C) for 36 hours affected the storability of corn (*Zea mays* L) seeds. In this case germination decreased by 70% for seed primed at 20°C and stored at 25°C for 12 months when compared to seeds treated at 10°C and stored at 15°C, which exhibited a much smaller reduction (45%-20%) in germination over the same time frame (Chiu et al., 2002). The level of peroxide was found to be lower in these seeds compared to seeds primed at 20°C, which may be associated with enhanced antioxidative activities (Chiu et al., 2002). Similarly, Bradford (1986) found that priming seeds of tomato (*Solanum lycopersicum*) under low temperatures resulted in more rapid emergence compared to seeds primed at warmer temperatures.

In arid and semi-arid lands, key life processes are controlled by infrequent and irregular rain events which make revegetation success difficult to achieve with any

consistency (James et al., 2013). One of the essential goals in rehabilitating arid or disturbed areas is to provide and support the revegetation process within the shortest time practicable after restoration work has commenced to reduce economic costs and environmental damage (Burke, 2003). Seeds in restoration seedbanks may be stored for long periods of time and while in storage may undergo a level of deterioration due to many reasons (Bewley, 2013). Under these circumstances seed priming may improve the longevity or have a rejuvenating impact on aged seeds (Butler et al. 2009; Paparella et al. 2015). In addition, erratic seed germination and poor seedling emergence of wild species in the field is a particular challenge for restoration and unfortunately exceptionally common (Erickson et al., 2016 a, b).

The hydropriming of seeds is regarded as broadly applicable to enhancing germination performance of many species, particularly in the horticultural and agricultural sectors (Lutts et al., 2016). Consequently, there is potential for hydropriming to similarly enhance the germination of wild plant species that commonly display a reduction in uniform performance due to differences in genetics, seed maturity and dormancy prevalence (Baskin and Baskin, 1998; McDonald, 2000). This study aimed to investigate the effects of hydropriming on the germination of selected native species (*Cymbopogon obtectus* and *Eriachne mucronata*) and their performance under different levels of water stress. The two species are perennial native grasses (Poaceae) from the Pilbara region of Western Australia. In this context, we hypothesised that primed seeds will germinate more rapidly compared to non-primed seeds and show an enhanced capacity to resist water limiting conditions and germinate more readily at lower water potentials (MPa).

3.3 Material and Methods

3.3.1 Seed collection

Seed of *Cymbopogon obtectus* and *Eriachne mucronata* were collected in March 2015 and Sept/Oct 2014, respectively by commercial seed collectors on behalf of BHP Billiton. Following collection seeds were stored in a refrigerated cool room for several months (~5°C and 50% RH). Seeds were then sent to Kings Park for storage in a seed drying room (15°C and 15% RH) until utilised in this study. For experimental purposes seeds were initially cleaned from the covering florets by gently using a rubbing mat to break the florets apart and then the seeds were separated from the remaining florets and chaff by the use of sieves and vacuum separation ("Zig Zag" Selecta, Machinefabriek

BV, Enkhuizen, Netherlands). Once cleaned, seed fill was then assessed using a Faxitron Seed Imaging System (MX-20 digital X-ray radiography system, Faxitron, Tucson, AZ, USA; Kodym et al. 2010) by examining seeds where the presence of a white/grey internal structure was considered filled.

3.3.2 Priming experiment

Initial germination testing (Chapter 2, section 2.3.4) commenced in November 2015 with seed priming experiments using water (Hydropriming) commencing in April 2016. The process started by preparing three replicates of four mesh bags containing 50 seeds/bag for each of the different priming treatments. Priming treatments consisted of placement of seeds in aerated water for 3 h, 6 h, and 9 h at room temperature (~23°C) to compare against a control group of seeds which were left unprimed. The priming approach involved placing the bags with seeds in the priming cylinders (2.5 L) which were filled with water, with plastic tubing connecting the priming unit to an aquarium air pump (HAILEA, Model HAP-60). This provided a continuous supply of air for the entire duration that seeds were primed.

After priming, seeds were removed and left to dry for five days in the seed drying room (as previously described). Following drying seeds were then surfaced sterilized in 2% (w/v) calcium hypochlorite ($Ca[OCl]_2$) solution for 30 minutes with the addition of a vacuum to improve the surface sterilisation process (10 minutes on-off-on, at -70kpa) and then rinsed four times in sterilized de-ionized water. Seeds (each treatment of priming duration) had three replicates, each replicate had 200 seeds divided into four sections control, -0.25, -0.5,-1.0 MPa to test the effect of PEG) were then plated onto 90 mm Petri dishes lined with seed germination papers (Advantec, 84mm) moistened with solutions of either water (control) or polyethylene glycol-8000 solution (PEG 8000, Sigma-Aldrich-USA) prepared to generate a range of different water potentials (0, -0.25, -0.5, -1.0 MPa) at 23°C according to the methodology described by Michel (1983). Petri dishes were tightly wrapped with plastic film to reduce moisture loss and then incubated at 30°C (Li et al., 2011). The incubator (Thermoline Scientific) was set to a 12/12 h alternate light and dark cycle (white fluorescent tubes, 30 W, with a photon flux density of ~30 μmol m^{-2} s^{-1}, 400-700 nm) with seeds left exposed to these conditions.

Seeds were scored daily for the first week then weekly for an additional three weeks with germination defined as radicle emergence greater than one-third of the seed coat length. Time to 10% and 50% of the maximum germination was also calculated using

germinator software (version 2010). Germination data were analysed using regression analysis and Kruskas Wallis to check the association among the variables and their impact on the dependent variable in GenStat version 18 (Copyright 2016, VSN International Ltd); no data transformation was needed. Significant differences between treatments were detected by using Fisher's LSD as the multiple comparison tests ($P<0.05$).

3.4 Results

3.4.1 Germination percentage

Germination percentage in *Cymbopogon obtectus* without priming was not affected by mild drought stress (-0.25 MPa); however, under medium to severe drought conditions (-0.5 MPa, -1 MPa) the germination of non-primed seeds decreased significantly ($p<0.05$).

Priming seeds for 3 h improved germination significantly under severe stress conditions (-1 MPa) to 41% compared to the germination in seeds which were not primed (<10%) but subjected to the same severe water stress ($p<0.05$; Figure 3.3.a). Priming seeds for 9 h increased germination significantly to 47% compared to non-primed seeds under severe stress conditions Similarly, priming seeds for 6 h improved germination significantly under severe stress conditions (-1 MPa) when compared to the germination of seeds which were not primed but subjected to severe water stress. Six hours of priming was found to be more effective for enhancing seed tolerance to moisture limiting conditions compared to all the other priming treatments assessed as germination increased significantly to 86% at -1 MPa ($P<0.05$; Figure 3.3.a).

In comparison, the germination of *Eriachne mucronata* seeds did not change when exposed to any of the water stress conditions assessed. Similarly, none of the priming treatments proved to be effective in increasing the overall germination percentage in this species ($p>0.05$; Figure 3.3.d).

3.4.2 Germination rate

The germination rate of *Cymbopogon obtectus* in some priming treatments was more rapid when compared to untreated seeds ($p<0.05$; Figure 3.3.b). Priming seeds for 3 h and 6 h significantly reduced the germination time under mild water stress condition (-0.25MPa) ($p<0.05$).

However, priming seeds for longer (i.e. 9 h) did not affect the rate of germination when compared across the same water stress ($p>0.05$; Figure 3.3.b,c). Under medium water stress (-0.5 MPa) priming treatments for 3 h was significant in increasing the rate of germination when compared against the control treatment ($p<0.05$). Under severe water stress (-1 MPa) all priming treatments significantly enhanced the rate of germination though the priming duration of 6 h proved to be more effective than the other two priming treatments ($p<0.05$; Figure 3.3.b,c).

Priming seeds of *Eriachne mucronata* for 3 h, 6 h and 9 h did not significantly reduce t50 when compared to untreated seeds under normal conditions ($p>0.05$; Figure 3.3.e). In comparison, priming seeds for 3 h was significant ($p<0.05$) in reducing the t50 under mild water stress (-0.25 MPa) while priming seeds for 9 h then exposing them to severe water stress (-1 MPa) proved to be effective in reducing t50 when compared to all the other treatments assessed.

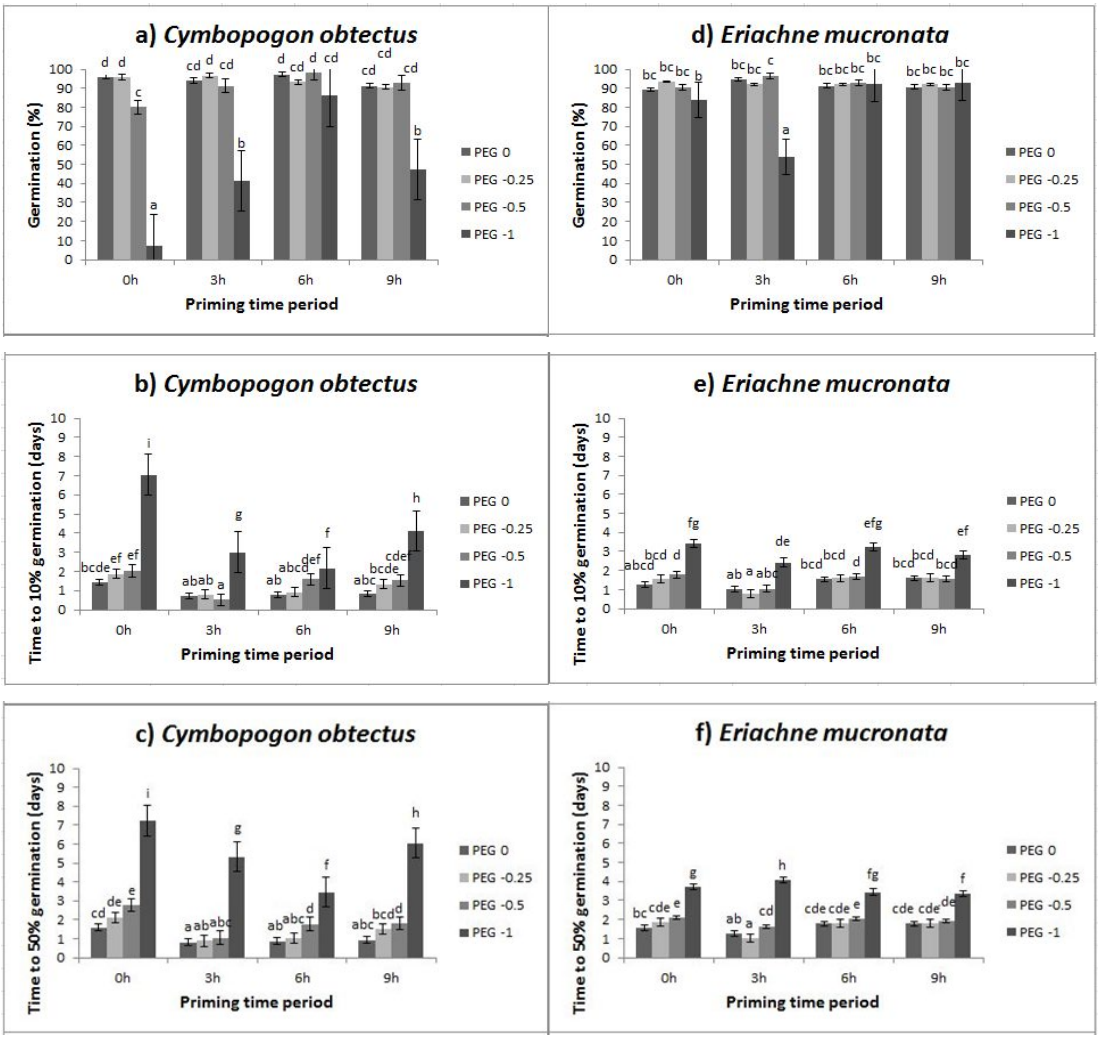

Figure 3.2. Germination percentage (Mean ± s.e.), time to 10% germination and time to 50% germination of *Cymbopogon obtectus* and *Eriachne mucronata* seeds after different priming and incubation treatments.

3.5 Discussion

The seedling stage is one of the most sensitive periods in the lifecycle of a plant. The amount of time required for seeds to germinate is different depending on the species, with the amount and distribution of rain affecting different species in different ways (Gutterman, 2012). The semi-arid Pilbara climate receives occasional tropical rainfall (average ~300 mm annually) with high evaporation which is particularly extreme in summer (Bureau of Meteorology, 2011). Hydropriming may hydrate seeds without using chemicals and improve germination in *Cymbopogon obtectus* and *Eriachne mucronata* and enable them to complete their life cycle under extreme arid conditions. Based on the results presented priming can improve germination and reduce the time required for germination to occur, potentially offering tangible benefits when used in broad scale restoration programs.

Germination percentage and germination rate are used to compare and rank germination of seeds under different treatments and environments (Brown & Mayer, 1988). Seeds of *Cymbopogon obtectus* had high germination under fully hydrated conditions and also under mild water stress (-0.25 MPa), which significantly decreased under medium to high moisture stress conditions (-0.5 MPa and -1.0 MPa) ($p<0.05$). This result may indicate that *Cymbopogon obtectus* is somewhat vulnerable to drought at this point; however, germination was improved and in particular tolerance to medium water stress (-0.5 MPa) after priming seed for 6 h which indicates that the hydropriming treatment was successful and improved germination under suboptimal germination conditions. Even under severe drought stress (-1.0 MPa) the germination of *Cymbopogon obtectus* seeds was nevertheless improved after the application of a 6 h priming treatment raising germination from <10% in unprimed seeds to >80% in the 6 h primed seeds.

Germination percentage of *Eriachne mucronata* seeds without a priming treatment was high in water and did not decline with increasing water stress (-0.25 to -1 MPa). This may indicate that *Eriachne mucronata* seeds are both highly tolerant to severe drought conditions and that the limited range of water potentials assessed in this study were clearly inadequate for identifying the critical moisture thresholds where germination is significantly reduced and where priming treatments may be of some benefit.

The germination rate (i.e. the number of days to reach 10% (t10) or 50% (t50) germination) of *Cymbopogon obtectus* seeds was significantly improved through the use of seed priming with the best germination obtained by priming seeds for either 3 h or 6 h. In comparison, the 9 h priming treatment was not significant for germination rate compared to the control treatment which may indicate that the longer priming period (9 h) for this species may have been deleterious to the seeds. Indeed, germination for this species is usually very rapid with a t50 of only 1 day under standard germination conditions (i.e. 25°C) (S. Turner unpublished results). Given that *C. obtectus* seeds germinate quickly (<24 hours) it is highly likely that some had already commenced germination after 9 h priming so had lost desiccation tolerance by this point and were possibly injured when removed from the priming solution and redried.

All the priming treatments assessed for *Cymbopogon obtectus* significantly reduced germination time under mild drought stress (-0.25 MPa) ($p<0.05$). This may illustrate that even though final germination percentage was high under drought conditions seeds still benefited from the application of the priming treatment to reduce germination time.

Under medium water stress conditions (-0.5 MPa) the best priming period found for *Cymbopogon obtectus* seeds was 3 h as it provided high germination as well as reduced the time to germinate. The longest time period to germinate was observed for the seeds under severe drought stress (-1 MPa) that were not exposed to any of the priming treatments prior to the imposition of the water stress. By comparison, seeds that were primed had increased germination speed when exposed to water stress with the 6 h priming treatment proving to be particularly effective.

The time to 10% germination (t10) indicates early germination of seeds. Under fully hydrated conditions *Cymbopogon obtectus* seeds germinated to a similar level regardless of whether seeds were primed or left unprimed. However, under mild water stress conditions (-0.25 MPa) some of the primed seeds (3 h and 6 h) had significantly enhanced germination speed that may indicate that these treatments were near optimal for enhancing drought stress. Under medium water stress (-0.5 MPa) priming for 3 h reduced the t10 significantly when compared to the other priming treatments which were not significant and consequently provided no tangible benefit. Under severe water stress (-1 MPa) all the priming treatments significantly reduced the time taken for germination to occur when compared to the control treatment. The results of this study indicate that priming is effective in increasing early germination of *Cymbopogon obtectus* under arid conditions.

The germination rate (t50) for *Eriachne mucronata* seeds was also significantly improved by priming in some treatments. Results show that priming seeds for 3 h reduced the time taken to attain 50% germination under mild to medium drought stress (-0.25 MPa and -0.5 MPa) when compared to the control treatment as well as to the other priming periods assessed as part of this study. However, under severe drought stress (-1 MPa) priming seeds for 9 h provided better results suggesting that a longer priming period may be beneficial when seeds are sown and exposed to severe water stress and of more benefit under variable field conditions.

The time taken to achieve 10% germination (t10) of *Eriachne mucronata* seeds did not differ significantly ($p>0.05$) across all the priming treatments assessed under fully hydrated conditions. However, under all drought conditions the 3 h priming duration was beneficial in enhancing the germination rate (t10) with priming for a longer period (6 h or 9 h) not showing any clear benefits. Results indicate that priming may have some positive effects on germination parameters such as total germination and germination speed for seeds under certain water stress. Similar results were found in a study using hydropriming (unlimited amount of water for 24 h) on two native species *Penstemon roseus* and *Castilleja tenuiflora*

in Mexico with germination rate accelerated by priming in both laboratory and field conditions without affecting germination capacity (Belmont et al., 2018) . Another study by Matthew et al. (2018) shows that priming seed of two native species *Poa fendleriana* and *Pseudoroegneria spicata* in -1.5 to -2.5 MPa for up to 12 days improved germination rate with day to 50% emergence for primed seeds from 66.2 to 82.4% faster than non- treated seeds. The shortened emergence time under water limited conditions observed in this study is consistent with those of previous studies and highlights the potential benefits of adopting priming technologies for enhancing germination and emergence of wild seeds in land restoration programs (Casenave and Toselli, 2007; Casenave and Toselli, 2010; Matsushima and Sakagami, 2013; Li et al, 2017).

In conclusion, the germination percentage and rate of the two primed seeds showed significant improvement which can be applied to broader practices in arid lands. Priming enhanced the seeds capacity to resist water limiting condition and germinate more readily at lower water potential (MPa).

Chapter 4 Analysis of the functions of botanic gardens to improve their effectiveness in conservation and restoration

4.1 Abstract

Botanic gardens are aesthetic places that include several different plant collections. An increasing function of botanic gardens that has increased in recent times is their role in plant conservation due to the high risk of extinction around the world. Botanic gardens also attempt to increase the awareness of local communities about conservation issues and improve environmental attitudes of the public at large. This study aimed to identify the important structures and roles of botanic gardens that are necessary to accomplish this mission and how to best convey the importance of conservation to the general public. A survey was conducted that consisted of a series of multiple choice and short answer questions. The questions gathered information relating to what botanic gardens do in regards to conservation and the difficulties that they may currently experience which can limit their programs and could be avoided or overcome in future. The survey engaged botanic gardens of different sizes from different continents and climates in order to cover a broad and representative sample. The responses to the survey show that the majority of botanic gardens are committed to plant display, education, conservation and research. Most are open to the general public for visits and offer a range of services to encourage visitors including education facilities. The majority of botanic gardens rely significantly on volunteers as a way to engage people to undertake volunteer work such as guides, tour leaders and general assistants supporting several different aspects of the botanic gardens. The majority of botanic gardens display their plant collections thematically and geographically, however they may use other display groupings on occasion as well. Most of the botanic gardens hold between 2,000-5,500 plant species with the majority holding between 1,000-10,000 accessions. Most hold their accessions in seeds banks with the highest plant percentage held by botanic gardens being native species. The majority of botanic gardens receive from $100,000 to $10,000,000 AUD per annum derived from different funding sources, though the majority of funding was from the government and entry fees. The money that botanic gardens receive is in the majority of cases used to fund staff (50-75% of all funds received), with less than 25% of their budget spent on other functions such as horticultural displays, conservation and education. The highest focus of

botanic gardens is on research and conservation with the principle interests in taxonomy, ecosystem ecology, species recovery, horticultural research, restoration ecology and seed conservation. Most conservation programs undertaken by botanic gardens were focussed on *ex-situ* conservation (74%) while less (39%) were involved in in-situ conservation. Most botanic gardens that hold plants for conservation purposes have between 10 and 100 species being used in research programs. Several techniques are used for ex-situ conservation; the majority use seeds banks (38%) and field gene banks (31%), while DNA banking, in vitro storage and pollen banking were less popular with the majority of botanic gardens storing their seeds for the medium term. In general, all botanic gardens have various collaboration programs with different institutions such as other botanic gardens, national parks, universities and NGOs (non-government organisations). The majority of botanic gardens stated that ecosystem conservation and native plant conservation are their highest priority in conservation while propagation and cultivation were the most popular approaches for plant conservation compared to data stewardship. It was found that most botanic gardens manage their collection through the use of nurseries, glasshouses and seeds banks. Difficulties faced by botanic gardens due to budget limitations were centred on a lack of staff, research space and research facilities.

4.2 Introduction

The evolution of botanic gardens has developed over many hundreds of years with a gradual increase in knowledge as civilizations have developed and evolved (Spencer and Cross, 2017). The interest in plants started from the very earliest stages of human development. As an example, in Egypt the medicinal use of various plants dates back thousands of years to the Third Dynasty (2667-2648 BCE) which was documented through several papyrus manuscripts which still survive today (Spencer and Cross, 2017). Similarly, in Mesopotamia, clay tablets have been found that describe the royal palace garden of King Ashurbanipal II (668-627 BCE) where plants were precisely recorded and listed as either “materia medica” or “herbal”, which is considered by the botanical historian Alan Morton as “the earliest truly botanical work at present known” highlighting the importance that plants have held in different civilisations for thousands of years (Morton 1981).

An early definition of botanic gardens was proposed in ancient times by the Greek philosopher Theophrastus (372-287 BCE) who referred to botanic gardens as ‘a living plant collection established for an enquiry into plants’ (Forbes, 2016). In Europe during

the Middle Ages botanic gardens were linked to monasteries where the plants that they contained were commonly used as herbal remedies to cure and sooth various ailments and diseases (Heyd, 2006). During the 16th and 17th centuries, physics gardens became the earliest botanic gardens in the Renaissance Period with their main function being to increase knowledge about plants, and these gardens were mainly attached to universities where they were utilised for education and research purposes (Johnson, 2011). One of the first described physics botanic gardens was Padua Botanic Garden in Italy (1545). Shortly thereafter, this idea began to expand to the rest of Europe to places such as Zurich (1561), Lyons (1564), Montpellier (1598), Paris (1640), The University of Oxford (1673), and Chelsea Physic Garden (1673) (Rhodes, 1984). Physics botanic gardens during that period were relatively small and limited spaces incorporating familiar geometrical design, but during the 18th century physics botanic gardens expanded in space and species diversity as they were increasingly used for the pursuit of different botanical sciences such as taxonomy, plant anatomy and plant physiology (Johnson, 2011). Beginning during the late Renaissance, botanic gardens were used for popularising new and novel plant species such as tulips, orchids and palms as well as the acclimatisation of economically important plants from distant countries to establish new crops to break various monopolies held by countries such as Brazil (i.e. rubber - *Hevea brasiliensis*) the Netherlands (i.e. nutmeg - *Myristica fragrans* and cloves - *Syzygium aromaticum*), and China (i.e. tea - *Camellia sinensis*) (Wickham, 2012). In the middle of the 20th century a new role for botanic gardens rapidly developed due to the need to conserve threatened plant species as well as to increase awareness about plant ecology and the importance of plants in general (Borsch and Löhne, 2014).

Modern botanic gardens now incorporate conservation as an important and essential role (Dunn, 2017) (amongst other functions) with botanic gardens defined by the Botanic Gardens Conservation International (BGCI) as "institutions holding documented collections of living plants for the purposes of scientific research, conservation, display and education" (Wyse Jackson and Sutherland, 2000). However, while useful this definition may not be applicable for all botanic gardens around the world as many are little more than formally designed parks and gardens that serve very limited roles in supporting research and conservation initiatives. For example, the BGCI garden database contains over 3,400 registered institutions worldwide and while many of these may not fulfil certain criteria they are nevertheless recognized as having an important role in performing activities such as botanical resource management, botanical and taxonomic research,

horticulture development, conservation as well as education and extension (Wyse Jackson, 1999, Wyse Jackson and Sutherland, 2000, Sharrock et al., 2010).

Worldwide there are an estimated 500,000 plant species which includes angiosperms, gymnosperms, ferns, lycophytes, and bryophytes (Corlett, 2016). The number which are threatened or near threatened has not as yet been accurately assessed (Sharrock et al., 2014) with some studies attempting to predict the likelihood of extinction of certain groups such as orchids, and palms as these are far better taxonomically described and their biogeography better understood (Arroyo-Rodríguez, 2007; Swarts and Dixon, 2009). For example, a recent study by Pimm and Joppa (2015) suggested that up to a third of all angiosperms might become extinct with the majority of these species not as yet described. The Millennium Ecosystem Assessment (2005) claimed that large irreversible losses in the diversity of life have been more rapid in the last 50 years than ever before due to various human actions and highlighted five major threats for biological loss. These major threats include habitat destruction, fragmentation, and degradation (Ter Steege et al., 2015). In Brazil, urbanization and agricultural expansion are considered principle causes of habitat destruction and as a consequence plant extinction is an increasing threat to more than 46,000 plant species present in this country (Costa et al., 2018). Other threats include overexploitation, invasive species (van Kleunen et al., 2015), climate change and air pollution (MEA, 2005; Corlett, 2016).

The updated Global Strategy for Plant Conservation (GSPC) agreed at the Convention on Biological Diversity (CBD) meeting in Nagoya in 2010 to include, as its first target for its 2020 objectives that “Plant diversity is well understood, documented and recognized” with a second objective being that “Plant diversity is urgently and effectively conserved”. In the context of these objectives, botanic gardens have an essential role in plant conservation globally and in recognizing and documenting plant diversity (Miller et al., 2004; Heywood, 2010; Sharrock, 2011).

Botanic gardens conserve plant diversity through many different approaches and techniques (Figure 4.1). These can be broadly divided into *in situ* conservation which includes ongoing monitoring and the protection of individuals and populations, and *ex situ* conservation which includes, seed banks, arboretum and tissue culture collections that can supply plant material for ecological restoration, reintroductions, and plant breeding (Hird and Kramer, 2013; Griffiths et al., 2014). The establishment and maintenance of living collections is the simplest strategy to conserve plants however plants are at risk of inbreeding and hybridization with other related species (Havens et al., 2016). For example,

horticulturists at the Yerevan Botanic Garden in Armenia found that using a phylogenetic system to arrange taxa may promote hybridisation between different though closely related species, which is clearly a problem when trying to produce genetically pure seeds from threatened species (Akopian, 2010). Living collections may also need extra space to include multiple individuals of large growing taxa (i.e. trees) that contain sufficient representative genetic diversity (Ensslin et al., 2015). *Ex situ* conservation using seed banks through drying and storing seeds under low temperature conditions is potentially a highly effective way to conserve genetically representative samples from a large number of species in a space efficient way that requires relatively little maintenance (Martyn et al., 2009). There are more than 1,750 seed banks around the world (FAO, 2010). However, the quality of the accessions stored across different seed banks varies significantly as some collections may not represent the genetic diversity of wild populations or the seeds within the seed bank may have low viability or stored under inappropriate conditions (Corlett, 2016). In addition, seed banks in general do not follow a specific standard for conservation of wild species (Hay & Probert, 2013) and the seed quantity that is stored may not be sufficient to support large scale restoration efforts (Merritt and Dixon, 2011). Other *ex situ* conservation strategies include cryopreservation in liquid nitrogen for long term storage of seeds, embryos and shoot tips; tissue culture collections and the storing of DNA samples or electronic DNA sequence information to aid in taxonomic studies (FAO, 2010). The DNA and tissue bank collection at the Royal Botanic Gardens, Kew contains over 48,000 samples of plant genomic DNA stored at -80°C and 10,000 silica dried tissue samples at room temperature from about 35,000 plant species (KEW, 2018). Around the world, botanic gardens have unique scientific tools and insights as well as expertise and equipment to aid and enhance plant conservation As an example, the Millennium Seed Bank at the Royal Botanic Gardens, Kew with its state-of-the-art *ex situ* facilities located at Wakehurst Place, West Sussex, in the UK has banked seed from over 14% of the world's plant species (Griffiths et al., 2014) and aims to conserve 25% of bankable species by 2020 (Sharrock and Wilson, 2014).

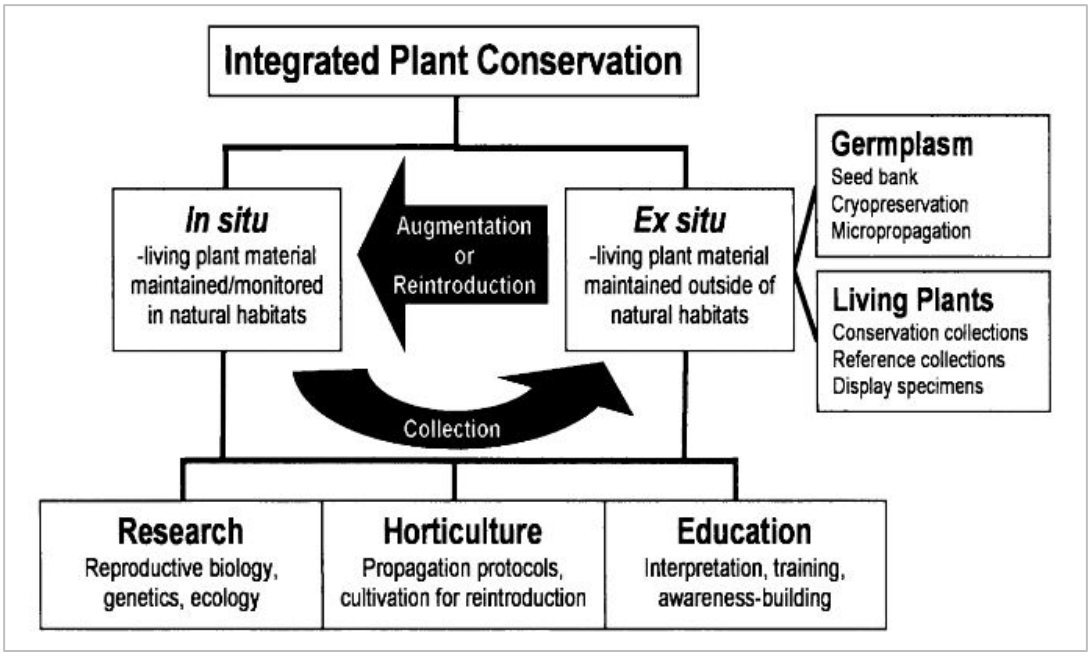

Figure 4.1. Overview of the different integrated plant conservation approaches
A schematic overview of the different integrated plant conservation approaches which integrates in situ *conservation of plants in their natural habitat with* ex situ *conservation techniques such as seed banking insuring that plant materials are available for research, horticulture and education activities as well as supporting the reintroduction of plants (when possible) and saving them from extinction if the continuity of* in situ *populations can no longer be guaranteed (Hird and Kramer, 2013).*

More recently, botanic gardens have developed a principle role for ecological restoration as they possess unique facilities and expertise needed to successfully undertake these types of activities (Crane et al., 2009; Miller et al., 2016). Various services that botanic gardens may provide for supporting ecological restoration include correct identification of plant species, appropriate planting techniques, identification and enhancement of plant-soil biological interactions, and the collection, cleaning, banking, enhancement and germination of diverse wild seeds (Hardwick et al., 2011). The Ecological Restoration Alliance (ERA) founded in 2011 by 15 leading botanic gardens that are active in ecological restoration aims to restore 100 damaged, degraded or destroyed ecosystems across the world (Abu Taleb et al, 2016). Examples of their work include; restoration of the Cloud Forest Sanctuary in Mexico by Jardin Botanico Francisco Javier Clavijero, restoration of Tallgrass prairie in Midwest USA by the Chicago Botanic Garden, restoring Forests in the East African uplands by Brackenhurst Botanic Garden, Restoration of the Huaruango woodlands in Peru by the Royal Botanic Gardens, Kew, Restoring Brazillian Atlantic Forest by Jardim Botânico Araribá, and recovery of the Florida Torreya by the Atlanta Botanical Garden (BGCI, 2018; ERA, 2018).

Networking between botanic gardens and other institutions is an effective tool for assisting plant conservation as it may reduce space restrictions, provides opportunities for establishing back up collections for the risk management of irreplaceable accessions (i.e. critically endangered species) and facilitates the transfer of knowledge and expertise between collaborating institutions (Donaldson, 2009). One of the largest global networks for botanic gardens is Botanic Gardens Conservation International (BGCI) established in 1987 by the International Union for the Conservation of Nature (IUCN). BGCI includes 800 botanic gardens spread across 118 countries with the principal aim to link botanic gardens and affiliated organizations and working together conserve and save threatened plants and ecological communities (Wyse Jackson and Sutherland, 2000). Another example of global networking and information sharing is the Nagoya Protocol in 2010 which is an international agreement for "sharing in a fair and equitable way the benefits arising from the utilization of genetic resources" (BGCI, 2012).

One of the most important elements in botanic gardens are living collections. It is estimated that around the world botanic gardens contain about 6.2 million accessions comprising more than 80,000 species (BGCI, 2012). Botanic garden display a wide range of plant diversity with many of them endangered and in some cases no longer surviving in the wild (Oldfield, 2010). Living collections in botanic gardens are commonly displayed in certain groupings such as geographical, taxonomical, thematical, horticultural, or ecological and serve one or more purpose such as supporting research, display, conservation and education (Dosmann and Groover, 2012). As an example, major display themes for living collections in the Australian National Botanic Gardens (ANBG) are taxonomic, ecological, geographical, and horticultural. Successfully achieving those groupings requires knowledge and expertise relating to plant ecology and biology as creating different groupings may require changes in certain environmental attributes, which is mainly done through changing characters indicative of each environmental zone such as temperature, precipitation, humidity and light (Beck, 2013) (Figure 4.2). For example, establishing rocky hills for displaying alpine plants in the Yerevan Botanic Garden required variable ecological conditions that have been modelled to reflect the conditions found in particular mountains in Armenia that are more suited to these types of plants (Akopian, 2010).

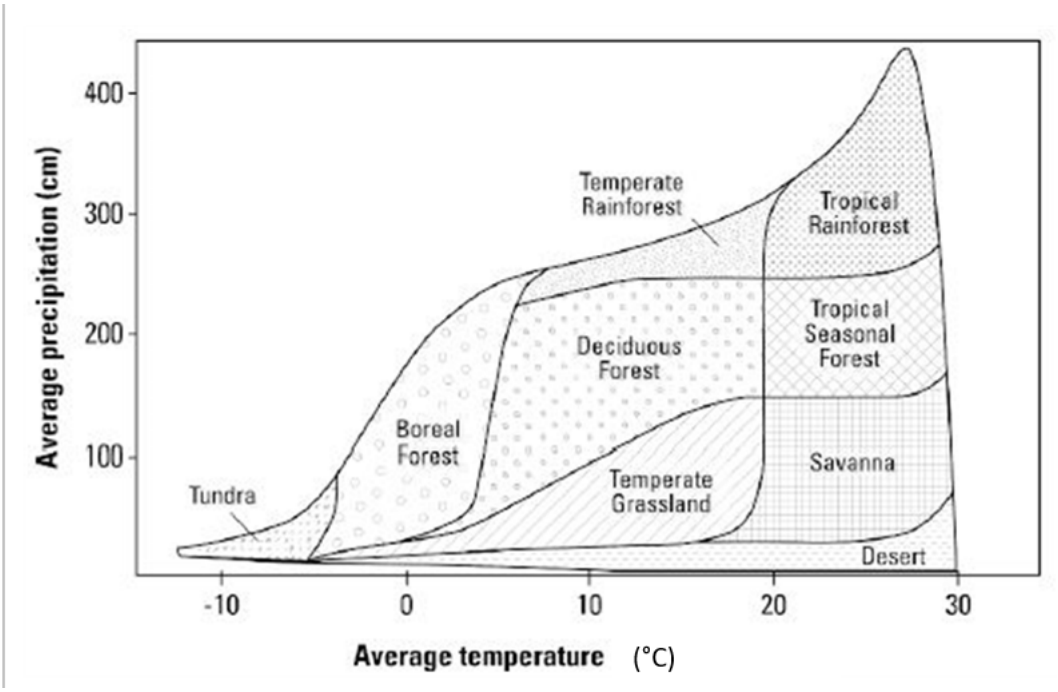

Figure 4.2. Global biomes in relation to temperature and precipitation *(Beck, 2013).*

As already discussed, botanic gardens are found world-wide (though there are relatively few in arid and semi-arid regions) and fulfil many important functions such as documenting the collection and taxonomy of living plants for the purposes of scientific research, conservation, display and education. A key feature of botanic gardens that has grown in importance in recent years is their role in supporting the conservation of endemic and endangered plant species and restoration of degraded habitats (Miller et al., 2016). There is a need for countries affected by desertification in arid and semi-arid zones to develop strategies to conserve and protect their endemic plant communities as well as to repair and restore those that have already been damaged and degraded. Further information is required to better understand strategies used by botanic gardens in conservation research to provide a framework for the future planning and design of botanic gardens to better serve their local region and communities. A survey was conducted of botanic gardens from different countries and climatic zones which aimed to collect and analyse information relating to their role and services to better assess their ongoing functionality and role in plant conservation.

4.3 Methodology

Targeted participants were botanic gardens from a range of different countries and continents. The study collaborated with the Botanic Garden Conservation International (BGCI), a membership organisation working with 800 botanic gardens in 118 countries

which is based in Richmond (near the Royal Botanic Gardens, Kew), Surrey in the United Kingdom to reach the greatest number of participants. The survey was conducted following ethics approval from Curtin University (HREC Project Number: HRE2017-0735), using Qualtrics Research Core (qualitative research software). The survey consisted of 30 questions including the consent question and a final question asking whether the respondents would be willing to share their information with BGCI to improve and enhance their GardenSearch database.

The questionnaire contained 30 multiple choice and short answer questions and where applicable an option was added (as other) in case the respondent wanted to expand and discuss their response in more detail. Survey questions were developed to collect basic demographic information about the botanic garden and then to divulge more detail about their role in plant conservation. The survey questions were based on the qualitative research style of several researchers (Kaczynski, 2004; Bringer, 2004; Wickham and Woods, 2005; Hutchison et al., 2009) and refined following the assistance of a qualitative survey expert (Amma Buckley, pers comm. 2018). Usually, more than one question was used to examine a single topic or theme. Questions 1 & 2 asked about the location of the garden, while questions 4 to 8 requested information about the size and structure of the garden. Questions 9, 10, & 11 asked about the plant species and the collections held by the garden. Questions 12, 13, & 14 asked about financial information and how it is divided amongst the different divisions and programs supported by the botanic garden. Question 15 asked about the botanic garden's education and extension programs. Question 16 asked if the botanic garden actively engaged volunteers and their roles within the botanic garden. Questions 17, 18, & 19 asked about research activities undertaken by the garden. Question 20 asked about collaboration programs between different organisations. Questions 21 to 26 asked about conservation and restoration activities undertaken by the garden. Questions 27 & 28 asked about information related to *ex situ* seed banking. Question 29 asked what they would consider adding to their botanic garden to enhance their conservation programs if they were in a position to do so. This last question was asked in order to identify and understand what each botanic garden finds important for supporting their conservation programs but cannot supply due to current budgetary constraints (Table 4.1).

In December 2017 the survey link generated through Qualtric software was distributed online through the BGCI online site under the news and event page (https://www.bgci.org/news-and-events/news/1443/). The link was also distributed through the BGCI electronic newsletter "Cultivate" that is sent regularly to botanic Garden

members. In February 2018 the survey was sent directly to select botanic gardens over a range of different countries and continents. A reminder was sent after one week after the survey was sent by email.

Qualitative data such as interviews and questionnaire were collected to understand and identify underlying reasons for the thoughts and opinions expressed for a small number of cases that could not be assessed using quantitative analysis to interpret the collected data (Suter, 2012). Therefore, data were thematically analysed (Bengtsson, 2016) by using the Nvivo software program to code and analyse open ended questions as well as closed ended questions. Qualtric analysis reports were used to interpret the results.

The time the survey was distributed was a limitation, being at the end of the year with several other surveys distributed by the BGCI at the same time. Also, many email addresses were not current and were returned back as faulty emails. Another limitation was that the survey was conducted only in English and thus non-English speaking participants may have been unable to respond due to the language barrier. It was noted that while many botanic garden participants had started the survey not all had finished with only about 50% of submitted surveys containing sufficient and useful information. Over 20 surveys generated a range of uncompleted responses that were rejected by the Qualtrics program and thus discounted from further analysis.

Table 4.1. Botanic Garden Survey Questions

	Question
1	Consent question (compulsory)
2	In which continent is the botanic garden located?
3	In which climate is your botanic garden located?
4	What is the total area of the garden?
5	What is the priority of the garden?
6	Is the garden open to the public? What is the annual visitation?
7	What facilities does the botanic garden offer?
8	How is plants displayed in the gardens?
9	What is the total number of plant species that the garden holds?
10	What is the number of accessions?
11	What are the numbers of species for living collection?
12	What is the annual budget for your garden?
13	Where has the garden received its funding over the last ten years?
14	Please indicates the percentage of how the budget is divided among the following area?
15	If public education is one of the activities, what are the facilities or techniques involved?
16	Does the Botanic Garden involve volunteer groups?
17	Is research and/or conservation a purpose of the garden?
18	What key research areas is the garden involved in?
19	What research is the collection based on?
20	Does the botanic garden participate or collaborate on projects with other gardens, groups, organizations?
21	Is the garden involved in conservation biology research?
22	What number of species within the living collection is presently part of a conservation research program?
23	What does your botanic garden consider a priority for land conservation?
24	What are the techniques used for ex situ conservation?
25	What is the aim of plant conservation from your garden perspective?
26	Is the botanic garden involved in a restoration program?
27	How long does the botanic garden seed bank maintain its seeds stores?
28	Have the botanic gardens developed the storage capacity?
29	If you had an unlimited budget what would you consider adding to your botanic garden to better serve your conservation work?
30	Would you like to provide the name of your Botanic Garden to update BGCI GardenSearch Records (Optional)

4.4 Survey Results

Questions 1 & 2 – Consent (compulsory) and in which continent is the botanic garden located?

Twenty-seven different responses were successfully lodged (i.e. provided consent so that their responses could be used as part of this study). The responses included botanic gardens from most continents where botanic gardens are found and from over 20 countries in total (Table 4.2). The majority of the botanic gardens that participated in the survey were from Europe (44% of responses). There were no responses received from any botanic garden from Africa (Table 4.2).

Table 4.2. Location of the 27 responding botanic gardens.

Continent	No. of Botanic Gardens	No. of countries
Africa	0	0
Asia	5	5
Europe	12	>8
Australia/Oceania	3	1
North America	5	4
South America	2	2
Antarctica	N/A	N/A
Total	**27**	**>20**

Question 3- In which climate is your botanic garden located?

The results show that most botanic gardens were located across a diverse range of climates (i.e. ten different climatic zones) with the majority (~26 %) located within an Oceanic climate (Table 4.3). Two climatic regions were not represented (Tundra and Polar ice cap).

Table 4.3. Climate in which the botanic garden is located (n=27).

Continent climate	No. of Botanic Gardens
Oceanic climate	7
Mediterranean climate	4
Humid continental	3
Desert	3
Rainforest	2
Humid subtropical	2
Tropical savanna	2
Subarctic climate	2
Monsoon	1
Steppe	1
Tundra	0
Polar ice cap	0

Question 4 – What is the total area of the garden?

The results show that the size of the botanic gardens ranged between less than 10 acres (4.0 hectares) to more than 1,000 acres (404 hectares). The most dominant size was 100- 1,000 acres (40-404 hectare) which accounted for 33 % (i.e. 9) of responses. (Figure 4.3)

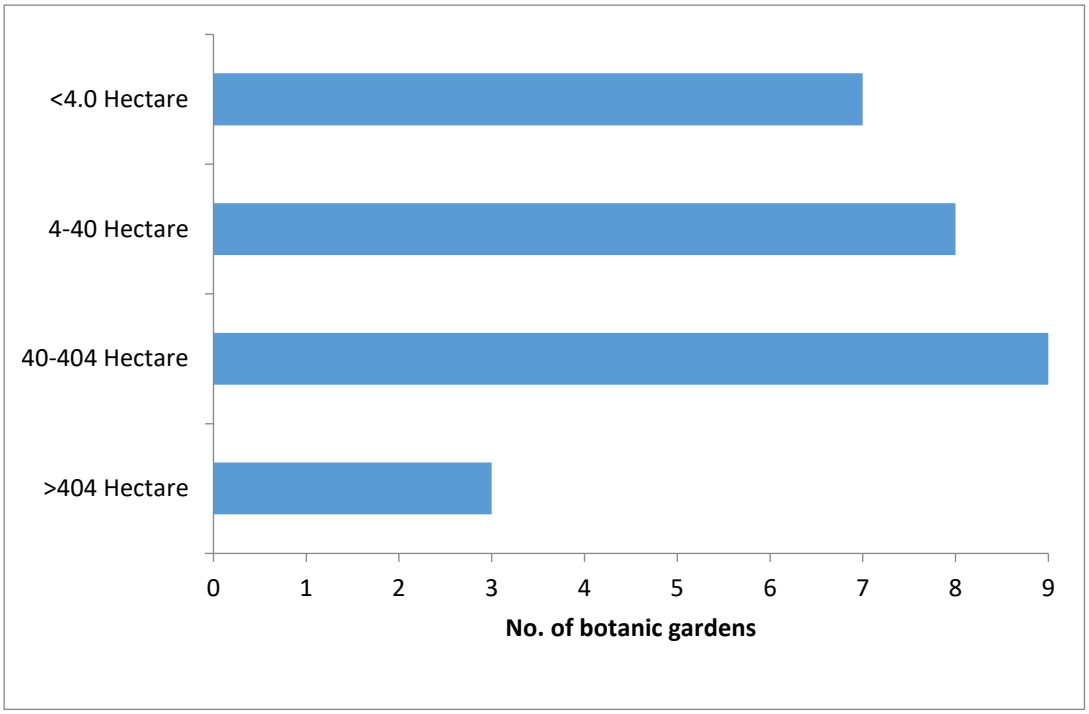

Figure 4.3. Botanic Garden land area size classes and the number of respondents in each (n=27).

Question 5 – What is the priority of the botanic garden?

Participants were asked to rank from most important to least important the various functions that their botanic garden may undertake. The results shows that the dominant priority based on this survey was education and plant display (44%), followed closely behind by conservation (30%), with both recreation (7%) and research (4%) ranked quite lowly (Figure 4.4). Some participants also added other priorities as well to their respective botanic garden such as tourism and propagation. This can be interpreted by weighing the priorities in order of importance by multiplying the number of botanic gardens by a weighing factor (5 for 1st priority, 4 for 2nd, 3 for 3rd, 2 for 4th, 1 for 5th); based on this it was found that highest priority was for education and display (83) followed by conservation (74), research (52), recreation (41) and finally heritage and archaeological display (38).

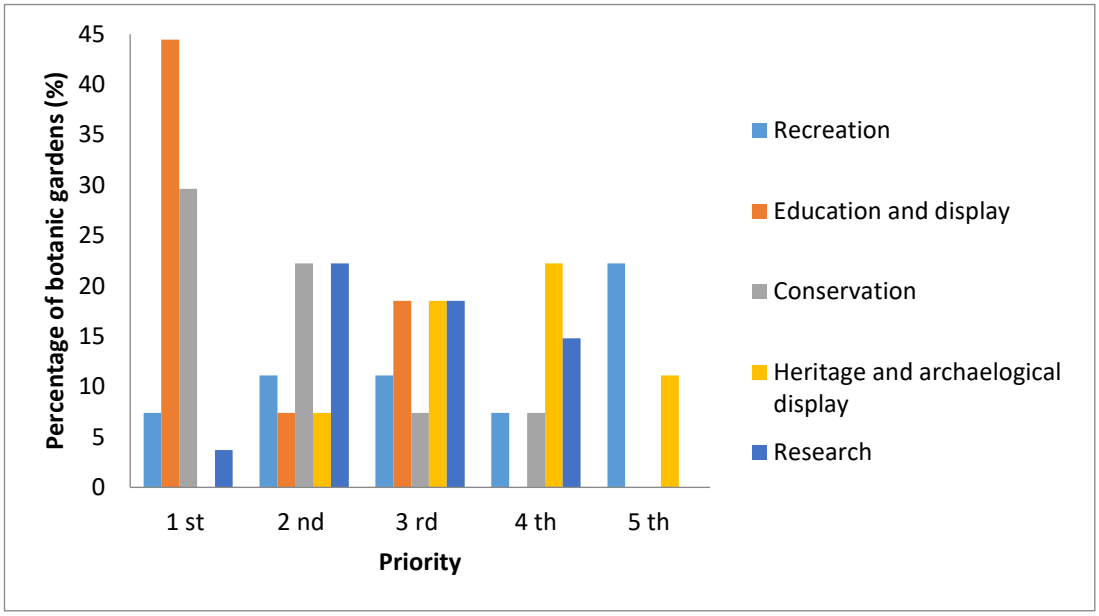

Figure 4.4. Importance ranking of different functions of Botanic Gardens.
Ranking from most (1st) to least (5th) important the functions of Botanic Gardens based on 27 different responses from the botanic gardens survey.

Question 6 – Is the garden open to the public and what is the annual visitation?

Participants were asked if the botanic garden is open to the public and the approximate number of annual visitations. Results show that most botanic gardens are open to the public (97 %) with only one closed to public visitations at present. Sixty seven percent of botanic gardens had at least 10,000 annual visitors (i.e. >190 weekly visitors on average) though 11 % (i.e. 3 botanic gardens) reported <1,000 annual visitors (Figure 4.5).

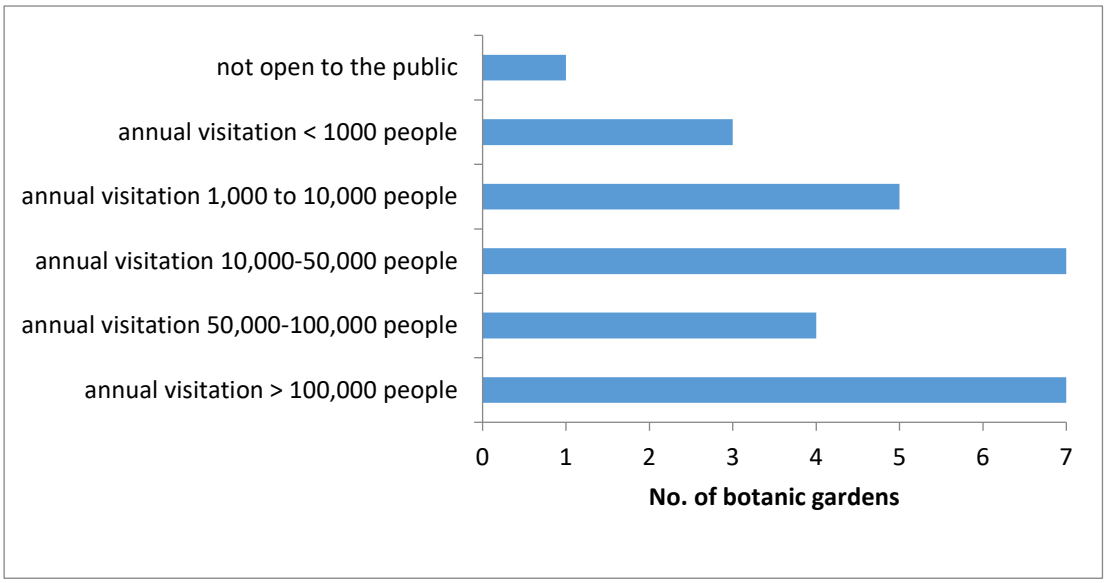

Figure 4.5. Public visitation classes based on responses from the botanic gardens survey (n=27).

Question 7 – What facilities does the botanic garden offer?

Participants were asked about the facilities that their botanic garden currently operates. A range of different facilities were listed with an additional space provided to include any other facility not mentioned elsewhere in the questionnaire. Results show that the majority (89%) of the responding participants contains conservation collections while automatic teller machines were found in only four botanic gardens. Additionally, some botanic gardens stated that they possess other facilities such as a Library, auditorium, palapa, and working space (Figure 4.6).

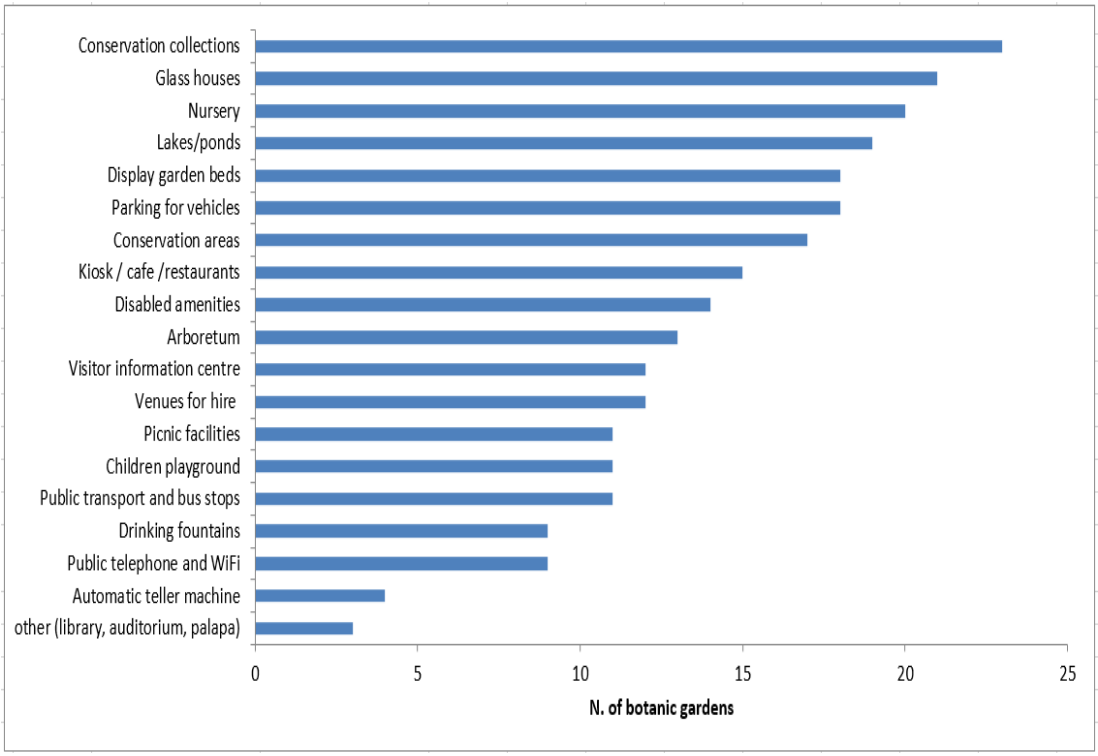

Figure 4.6. The range of botanic garden facilities provided based on responses from the botanic gardens survey (n=27).

Question 8 – How are plants displayed in the garden?

Participants were asked how plants were displayed in their botanic garden. The results from the survey showed that the majority of gardens display many of their plant thematically (23.1%) though overall there was relatively little difference (~ 7%) between the various categories (Figure 4.7). Additionally, one of the respondents added that parts of their plant displays were also grouped by habitat as well.

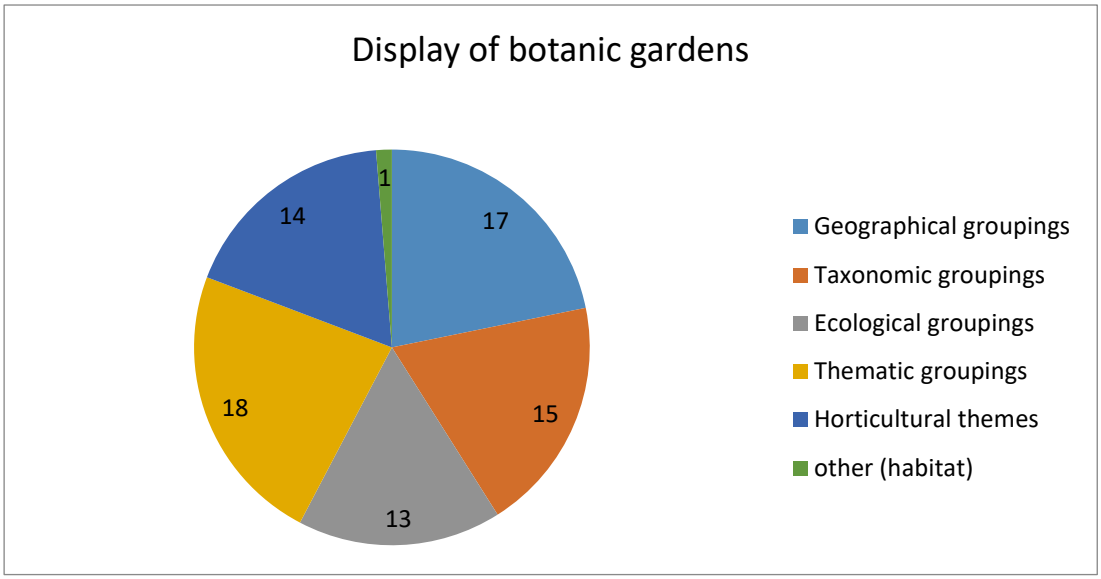

Figure 4.7. How plants are arranged and displayed in a botanic garden setting based on responses from the botanic gardens survey.

Question 9 – What is the total number of species that the garden holds?

Participants were asked about the number of plant species their botanic garden holds. Most botanic gardens (30%) hold more than 2,000-plant species in their collections while 11 % (3) reported holding more than 10,000 plant species (Table 4.4). In comparison, 26% reported holding < 600 plant species as part of their botanical collections.

Table 4.4. Number of plant species held by botanic gardens based on responses from the botanic gardens survey (n = 27).

Number of Plant Species	Percentage of responses (%)	No. of Botanic Garden
<600	26	7
600- 2,000	11	3
2,000-5,500	30	8
5,500-10,000	22	6
>10,000	11	3

Question 10 – What is the number of accessions?

The respondents were asked about the number of accessions the botanic garden held as part of their seed bank, nursery (i.e. containerised plants), and tissue culture collections, with multiple answers commonly provided. Results show that most of the 27 responding botanic gardens held accessions as part of their seeds bank (24) or as living accessions in a nursery environment (24) with only four reporting that they held some accessions as part of a tissue culture collection. Each botanic garden has a different number of accessions with the highest number of accessions class (>10,000) accounting for six of the seed banks and six of the nursery collections reported from different botanic gardens as part of this survey (Table 4.5).

Table 4.5. Number of accessions (as classes) and types (plants, seeds or tissue cultures) based on responses from the botanic gardens survey. The numbers in each column represent the number of botanic gardens answering yes to this question (n=27).

Number of accessions	Nursery based living collection	Seed bank accessions	Tissue culture collection
None	1	3	11
<10	0	5	3
10 to 100	4	4	1
100 to 1000	6	3	0
1000 to 10,000	7	3	0
>10,000	6	6	0
Total No. of botanic gardens	24	24	15

Question 11 – What is the number of species held in your living collection?

Participants were asked about the origins of the living species that their botanic garden held as part of their collections. The dominant answer was native flora while the least was cultivar flora. However, the percentage differs between the same living collections. As example, 33% of the botanic gardens answered that less than 25% of their living collection is native flora and 25% of botanic garden answered that their collection of exotic flora ranged between 50-75% (Table 4.6). Only 24 botanic gardens provided their actual percentage.

Table 4.6. Number of species for living collection (%) based on responses from the botanic gardens survey (n=27).

Percentage of living collection	Native flora	Exotic flora	Cultivar
less than 25%	33	17	50
25-50%	25	21	8
50- 75%	13	25	8
75-100%	20	20	4
Total (%) of BG that acquire the flora type	**91%**	**83%**	**70%**

Question 12 – What is the annual budget of your garden?

Participants were asked about the annual operating budget for their botanic garden. Results show that the majority (~60% of responses) of botanic gardens have an annual budget between $100,000 to $10,000,000 AUD while 3.7% have an annual budget higher than $100,000,000 AUD (Figure 4.8).

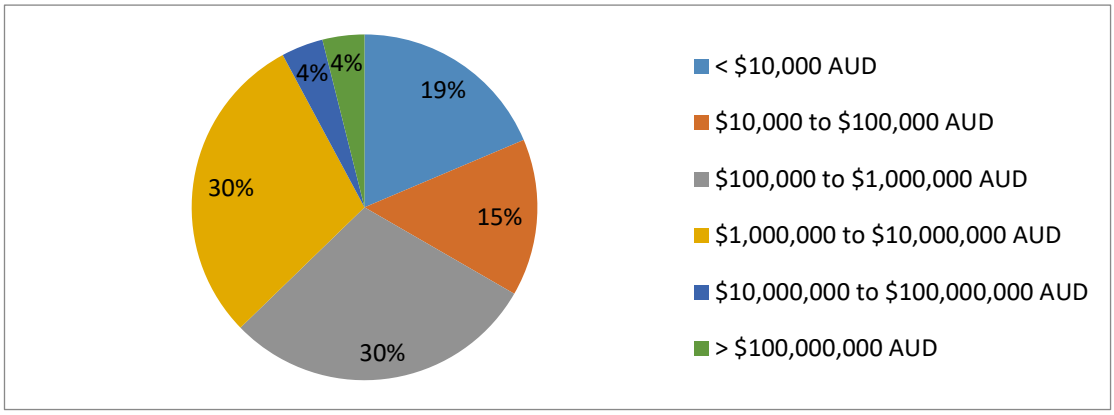

Figure 4.8. The percentage of botanic gardens falling into each of six budget classes (n=27).

Question 13 – Where has the garden received its funding over the last ten years?

The respondents were asked about the sources of funding for maintaining and operating their botanic garden, with multiple answers possible due to a potential mix of funding sources within the same organisation. The most common source of funding was government funding (29% of responses) while the least common form of funding was from nursery sales (8% of responses) (Figure 4.9). Other funding sources added by respondents included sponsorship, city projects, donations, university funding and consultancy.

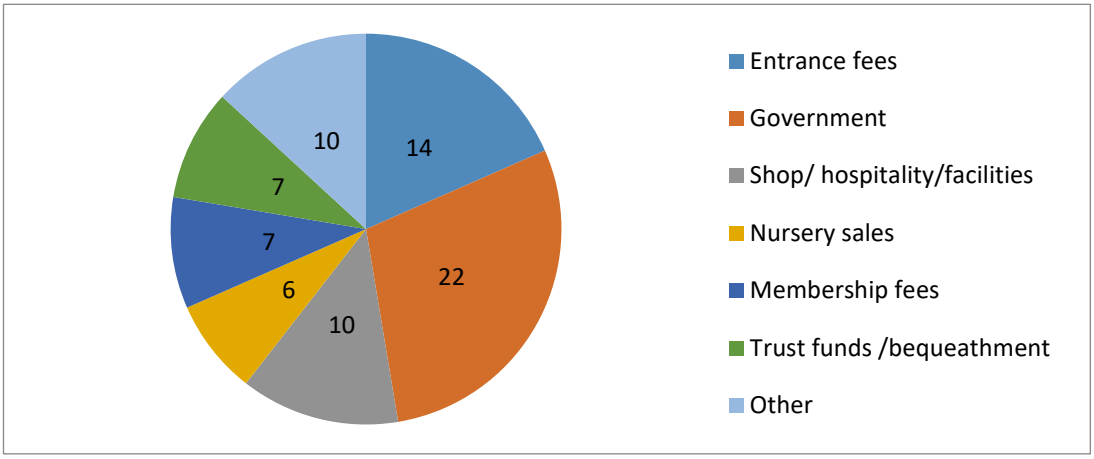

Figure 4.9. Main source of funding of botanic gardens over the last ten years (n=27).

Question 14 – Please indicate the percentage of how the budget is divided among the different areas?

Participants were asked how their budget was allocated across different areas. The percentage of the budget dedicated to certain areas was different between participants as shown in Table 4.7. Most of the responding botanic gardens (62%) dedicate 50-75% of their operating budget to staff salaries. While for horticultural display 81% of botanic gardens spend less than 25% of their budget supporting this activity. For all other budgetary areas including conservation, education, advertising, and visitor services 100% of respondents said that they dedicate less than 25% of their budget for supporting this activity. In addition, participants also added that other funding areas required support such as maintenance, rent for facilities, infrastructure, administration, insurance, research, operating costs, investment and repairs, energy costs, and information and communication technology. Only 22 participants provided a specific percentage to the allocated area. This can be interpreted alternately by weighting the percentage of allocation to each funding activities by multiplying the percentage by a weighting factor (1 for < 25%, 2 for 25-50%, 3 for 50- 75%, 4 for > 75%) and based on this it is found that highest allocation goes to staff salary (78.03) followed by horticultural displays (35.64) and then conservation, education, advertising and visitor, services at a weighting of 27.00 for each.

Table 4.7. Percentage allocation of funding to different activities based on responses from the botanic gardens survey (n=27).

Percentage of budget	Staff salary (%)	Horticultural displays (%)	Conservation (%)	Education (%)	Advertising (%)	Visitor services (%)
< 25%	12	81	100	100	100	100
25-50%	6	6	0	0	0	0
50- 75%	63	13	0	0	0	0
> 75%	19	0	0	0	0	0

Question 15 –If public education is one of the activities, what are the facilities or techniques involved?

Respondents were asked if public education is one of the activities that their botanic garden supports and what facilities or techniques are utilised to carry out this activity. Results show that most (24 responses) of the botanic gardens offer guided tours for visitors while only two botanic gardens provide computer terminals for general public use (Figure 4.10). Additionally, some botanic gardens responded that they offer other education facilities such as consultancy, community courses, mobile app., internet sites such as IrisBG Garden Explorer, and Schools Programs.

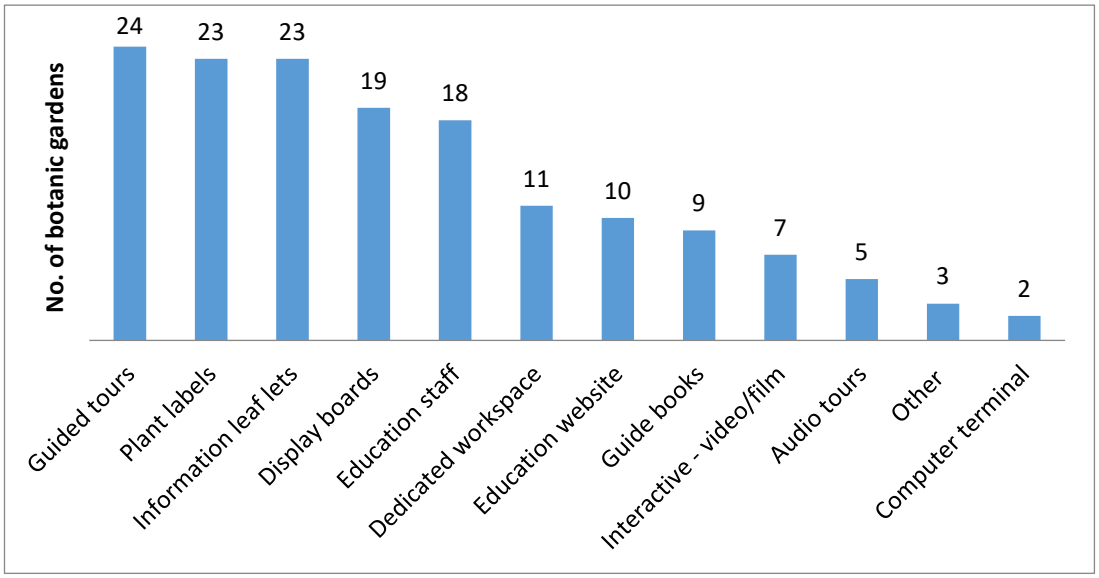

Figure 4.10. Number of botanic gardens supporting educational and extension activities based on responses from the botanic gardens survey (n=27).

Question 16 – Does the botanic garden involve volunteer groups?

Respondents were asked if their botanic garden supports volunteer groups and if so what roles they perform. The majority (67%) of botanic gardens have volunteer groups with volunteers undertaking many different roles such as running tours, assisting with greenhouse work, and helping with educational programs (Table 4.8). Thirty three percent of respondents (i.e. 8) stated that volunteers provided support for maintenance activities within their botanic gardens.

Table 4.8. Different volunteer roles undertaken in botanic gardens based on responses from the botanic gardens survey.

Volunteer roles	No. of gardens
Maintenance	8
Tour	3
Greenhouse	3
Education	3
General assistance	3
International project	2
Science	1
Endangered plant horticulture	1
Data encoding	1
Herbarium restoration	1
Reception	1

Question 17 – Is research and/or conservation a purpose of the garden?

Participants were asked if research and/or conservation activities are a purpose of the garden. The results show that the majority of botanic gardens (85%) consider research and conservation as one of their main purposes, while four of the respondents considered research and conservation to be low priorities for their organisations.

Question 18 – What key research areas is the garden involved in?

Respondents were asked to list the key research areas their botanic garden is involved in. More than one answer to this question was allowed. . Taxonomy was the most popular answer with 15 of respondents stating this was their most important function, with ecology (14), horticulture (13), and species recovery (13) also strongly represented. Other research areas that were added by respondents included conservation strategies, ethnobotany and archaeobotany. The least popular answer with less than 2 respondents was conservation biotechnology (Figure 4.11). The number of participants who answered this question was 23.

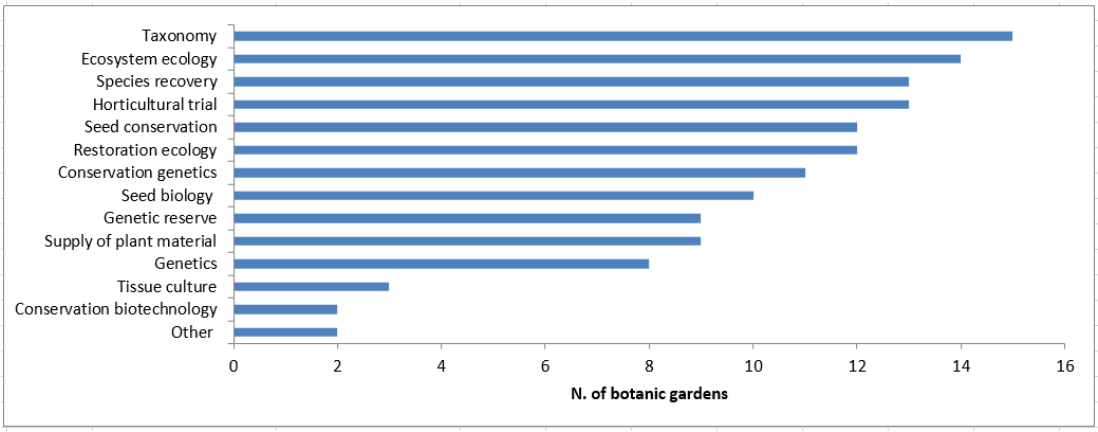

Figure 4.11. Priority research activities based on responses from the botanic gardens survey (n=23) with more than one answer allowed.

Question 19 – What research is the collection based on?

Participants were asked what research area their collection was based on. Three research areas were suggested (Taxonomic, Geographic, and Conservation) with an additional option provided if the suggested areas did not apply to their research collection. The results reveal that most botanic gardens (87%) base their collections on conservation research, while only 48% of respondents based their collections on geographic research (Figure 4.12). Additionally, one botanic garden responded by saying that ecological research is how they base their collection activities. The number of participants who answered this question was 23.

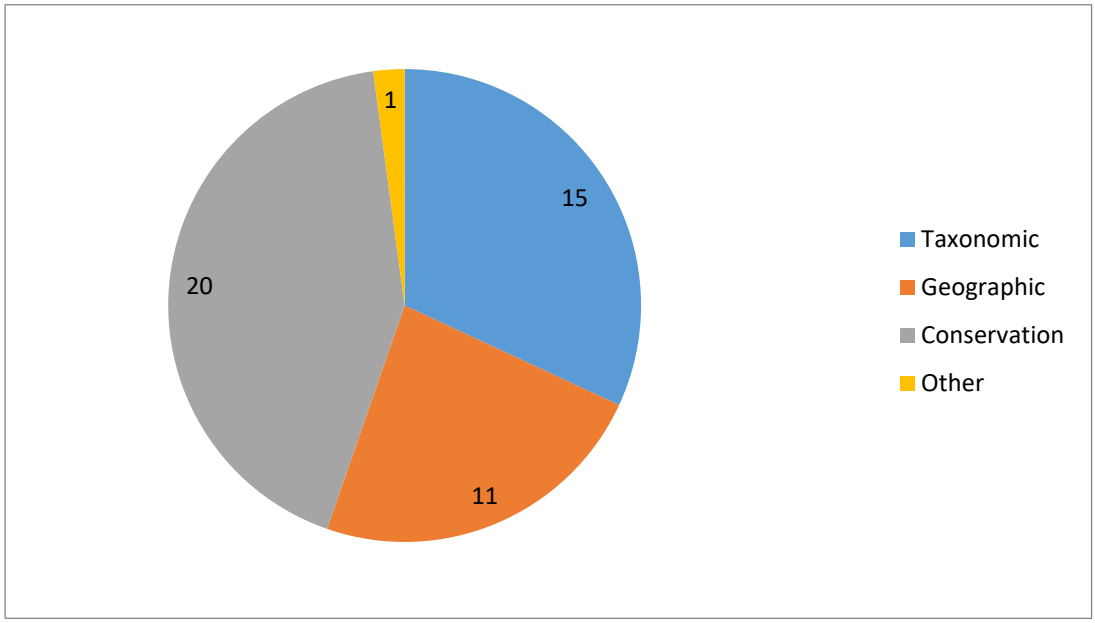

Figure 4.12. The research theme that each botanic gardens' collection focusses upon based on responses from the botanic gardens survey (n=23).

Question 20 – Does the botanic garden participate or collaborate on projects with other gardens, groups or organisations?

Participants were asked if they collaborate on projects with other gardens, groups or organizations and if so to define the type of collaboration activities they were involved with. Eighty three percent (n=19) of responding botanic gardens stated that they have collaborative projects of some form while four stated that they are not involved in any collaborative projects at present. Table 4.9 gives an illustration of the type of collaborations reported by 19 botanic gardens. Fifty percent of gardens collaborate with NGOs, for example the TCI Environmental Club, while universities, seed exchanges and other botanic gardens were also popular responses for other collaborating organisations (Table 4.9).

Table 4.9. A breakdown of different types of collaboration based on responses from the botanic gardens survey (n=19).

Collaboration type	No. of Botanic Gardens	%	Example of organization provided by botanic gardens
Botanic garden	4	25	Botanic Gardens Conservation International (BGCI)
Seed/Plant exchange	4	25	Mexican Association of Botanic Gardens (AMJIS)
National Park	1	6	Asociación Ibero-Macaronésica de Jardines Botánicos (AIMJB)
Universities	5	31	The Consortium of European Taxonomic Facilities (CETAF)
NGOs	8	50	TCI Environmental Club
Herbarium	2	12	United Kingdom Overseas Territories Conservation Forum (UKOTCF)
			Gothenburg Global Biodiversity Centre

Question 21 – Is the garden involved in conservation biology research?

Participants were asked if their botanic garden was involved in conservation biology research and whether this research involves *in situ* or *ex situ* conservation. The results show that the majority (61%) of botanic gardens are involved in *ex situ* conservation in some way while 40% are also involved in *in situ* conservation activities. The results also show that nine botanic gardens utilise both conservation approaches while four do not at present engage in either conservation activity. The number of participants who answered this question was 23.

Question 22 – What number of species within living collections are presently part of a conservation research program?

The participants were asked about the number of species that are currently a part of their conservation research programs. The dominant response (48%) was 10-100 species while 30% of responses stated that they were working on 10 or less species as part of their conservation programs (Figure 4.13). At the other extreme, four percent of responses stated that they were working on 1,000 to 10,000 species at present as part of their conservation research programs. The number of participants who answered this question was 23.

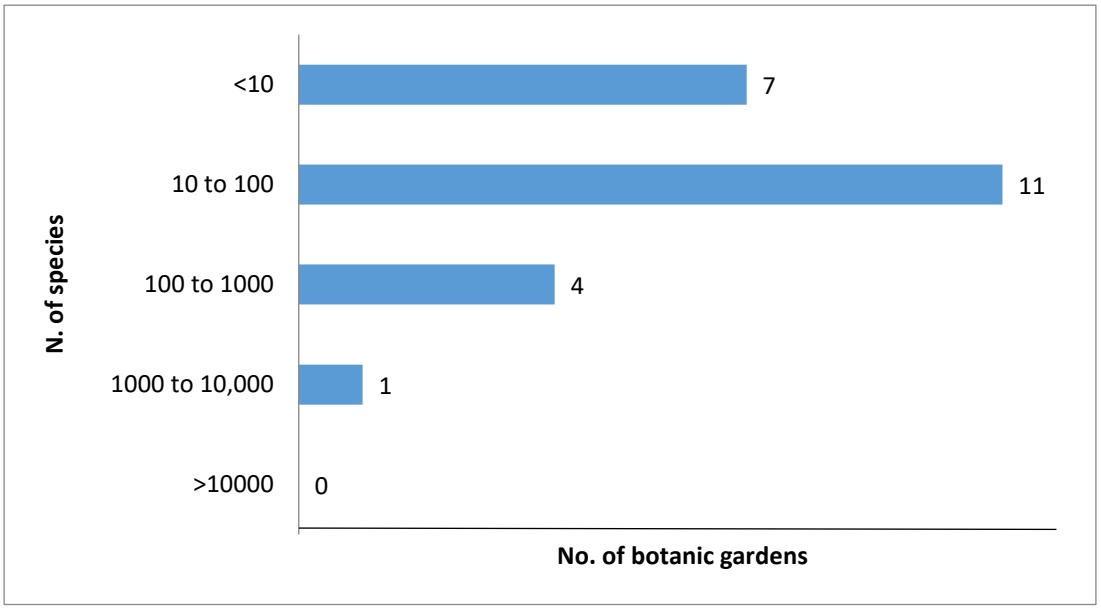

Figure 4.13. The proportion of species in conservation research programs based on responses from the botanic gardens survey (n=23).

Question 23 – What does your botanic garden consider as a priority for land restoration?

Participants were asked what they consider as a priority for land restoration research. Results show that 19 (70%) of respondents answered this question with eight providing no response. The two most popular response categories were ecosystem conservation (21%) and native plant conservation (21%) with the least popular responses being enhancing traditional management (5%), bioethical principles (5%), soil fertile layer preservation (5%), seed banks (5%) and climate change (5%) (Figure 4.14). The number of participants who answered this question was 19.

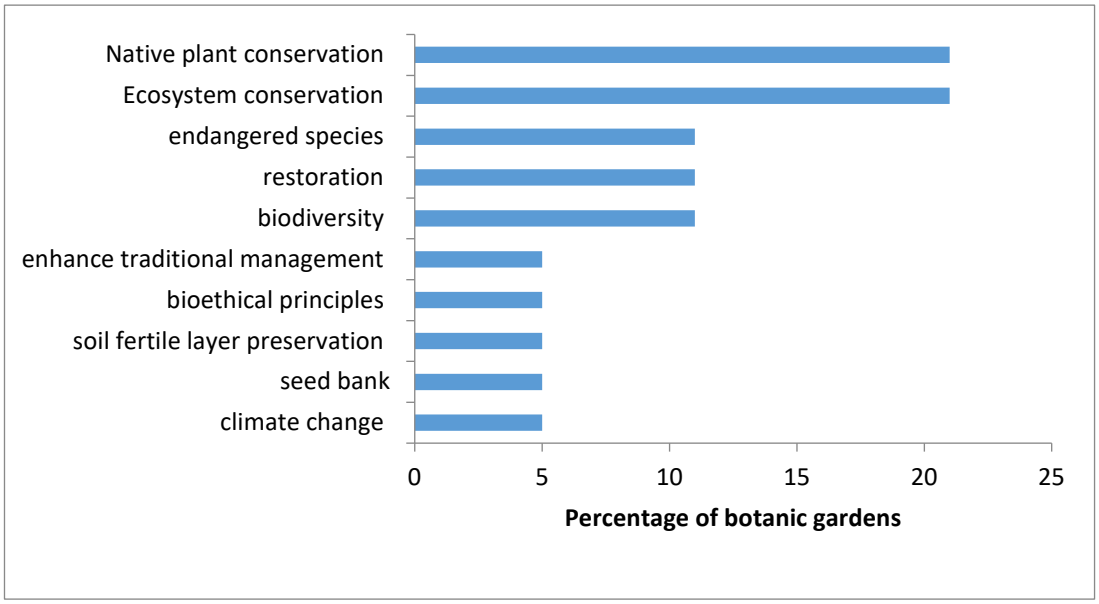

Figure 4.14. Priorities for land restoration based on responses from the botanic gardens survey (n=19).

Question 24 – What are the techniques used for ex situ conservation?

Respondents were asked about the different techniques they use for *ex situ* conservation, with multiple answers allowed. Of the 23 respondents, fifteen botanic gardens use seed banks, while the least popular technique was pollen banking (1 respondent). It was also stated by several botanic gardens that they use various plant breeding approaches and utilise living plants for *ex situ* conservation purposes (Figure 4.15).

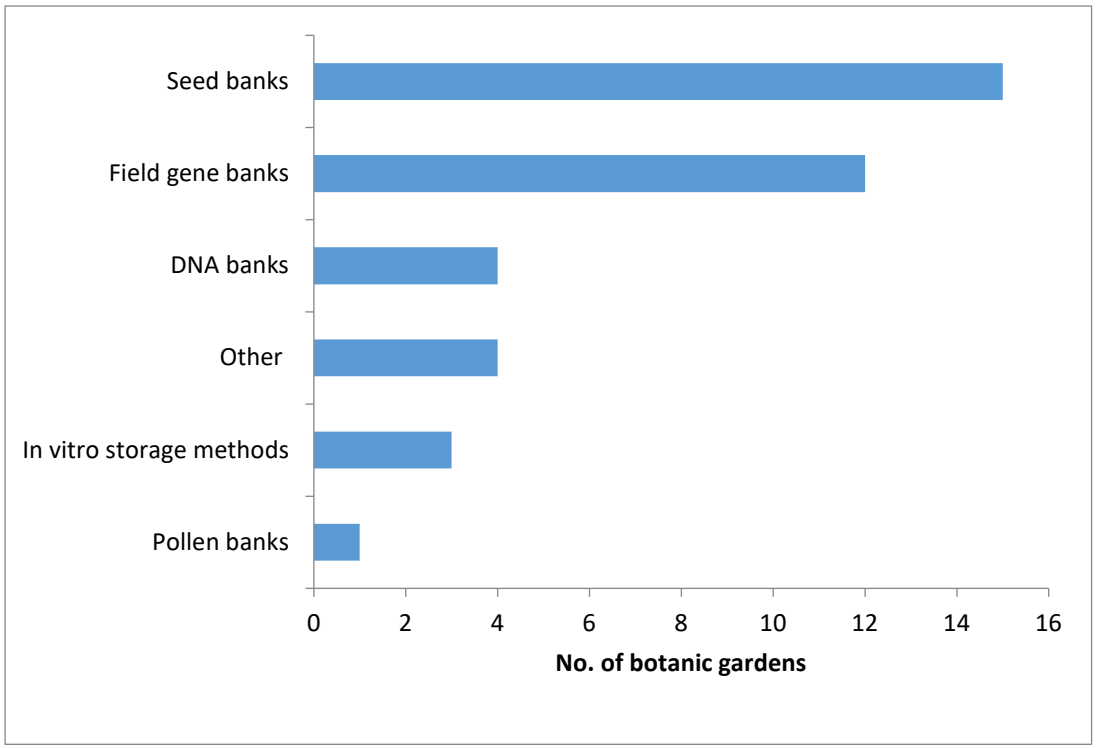

Figure 4.15. *Ex situ* conservation techniques based on responses from the botanic gardens survey (n=23).

Question 25 – What is the aim of plant conservation from your garden's perspective?

Participants were asked about the aim of plant conservation from their garden's perspective with multiple answers possible for question. The largest response was for plant propagation while the least popular response was data stewardship (Table 4.10). Other suggestions nominated by respondents to this question included maintaining biological diversity and integrative management of the landscape. The number of participants who answered this question was 23.

Table 4.10. Aims of plant conservation based on responses from the botanic gardens survey (n=23) with multiple answers allowed.

Aim of plant conservation	% of respondents	No. of Gardens
Propagation	28	19
Cultivation	26	18
Capture of genetic diversity	24	16
Data stewardship	19	13
Other	3	2
Total	**100%**	**68**

Question 26 – Is the botanic garden involved in a restoration program?

Respondents were asked if their botanic garden was involved in a restoration program. Of the 27 respondents, five botanic gardens have restoration programs dedicated to both the botanic garden itself and for restoration in other regions. Eight of the total botanic gardens have restoration program only for the botanic garden itself but not for other regions, five of the botanic gardens have restoration programs in other places or regions (but not for themselves), while nine of the botanic gardens do not have any restoration programs at present.

Question 27 – How long does the botanic garden seed bank maintain its seeds stores?

Participants were asked about the duration of storage for seeds in their seed bank. Of the 23 participants who answered this question, 35% of botanic gardens (i.e. 8) stated that they use medium term storage (25 years) as the basis for managing their collection. For long term storage (75 years) only 22% of botanic gardens responded by stating that they use this extended storage time. Two other respondents stated that they do not run and maintain their own seed banks; however, they do bank some of their seed in other seed resource centres operated by external organisations.

Question 28 – How have the botanic gardens developed their storage capacity?

The participants were asked about how the botanic garden developed their germplasm storage capacity with multiple answers possible for this question. The number of participants who answered this question were 19.The largest response (41%) was by using nursery or glass house facilities for growing and maintaining nursery plants while seed banking was the next most popular response (36%). Tissue culture and cryopreservation were the least popular approaches for the storage of *ex situ* germplasm (Table 4.11).

Table 4.11. Germplasm storage capacity of the responding botanic gardens (n=19) with multiple answers allowed.

Storage capacity	% of all responses	No. of responses
Yes - by nursery and/or glass house	41	16
Yes - by seed bank	36	14
No	10	4
Yes - by cryopreservation	8	3
Yes - by tissue culture	5	2

Question 29 – If you had an unlimited budget what would you consider adding to your botanic garden to better serve your conservation work?

Participants were asked an open ended question about what they would consider adding to their botanic garden if they had an unlimited budget. This question was asked to better understand what botanic gardens view as highly desirable/necessary for achieving their conservation goals; however, are unable to effectively achieve this due to budget limitations. The number of participants who answered this question was 20. Forty five percent of respondents stated that they would hire more staff for activities such as the collection of plants and plant propagation. Thirty percent stated that they would put addition money into better research facilities including seeds banks, DNA and Pollen Banks, and *in vitro* storage facilities. Twenty five percent would increase the size of some of their under-represented collections such as non-angiosperm collections, and wild and endangered species. Twenty percent stated that they would like bigger spaces including specialised nursery facilities, and general areas for growing live material. The development of better collaborations with other institutions (15%) and increasing study and research opportunities (10%) were relatively less popular (Table 4.12).

Table 4.12. A summary of botanic gardens responses to how they would enhance their conservation programs if they had an unlimited budget (n=20).

Suggestion of botanic garden	No. of responses	% of responses
More staff	9	45
Better research facilities	6	30
Increase collection	5	25
Bigger space	4	20
Better collaboration with other institutions	3	15
Increase research and study opportunities	2	10

Question 30 – Would you like to provide the name of your Botanic Garden to update BGCI GardenSearch Records (Optional)

Twenty botanic gardens answered yes to this question.

4.5 Discussion

The survey obtained responses from 27 botanic gardens from several different countries and most continents. The analysis of this information aimed to better understand the different functional aspects of botanic gardens and how they deal with plant conservation and implement and structure their research programs to support their core mission. Importantly, outcomes from this analysis will provide a better understanding of how best to plan and make insightful decisions through knowing what criteria are considered important and identifying the core functions of established botanic gardens. Capacity building centred on niche conservation and restoration programs to improve the management of degraded arid and semi-arid land and will no doubt improve these activities where resources are currently lacking.

The survey results showed that the majority of botanic gardens regardless of their location or size are deeply committed to plant display, education, conservation and research as their main priorities. These core functions of botanic gardens are what the world requires at present with the increasing risk of many plants species becoming extinct and the ongoing loss of many lands due to human activities. A key aspect of what defines most (in the case of this survey >90% of respondents) botanic gardens is being open and readily accessible to the public; visitors are essential for the success and popularity of botanic gardens due to their role in increasing peoples' knowledge about botany and improving their behaviour towards nature (Donaldson, 2009; Blackmore et al., 2011). A recent study highlighted the importance of botanic gardens in improving the knowledge and attitude of people towards plant conservation (Williams et al., 2015). The reach of botanic gardens is formidable in improving the public's perception of conservation issues with more than 11.8 million people recorded as visiting an Australian botanic garden in 2000 alone (Australian Bureau of Statistics, 2005) and over 300 million botanic garden visitors a year reported globally (BGCI, 2012).

Botanic gardens are motivated to invest significant effort to enhance their public appeal by providing a variety of facilities (Figure 4.6) to improve the overall visitor experience and the display of plant collections and flowers in well maintained public open spaces. The survey illustrates that botanic gardens contain many types of facilities to support their own work and enhance the experiences of visitors to the botanic gardens. Facilities and displays provided by different botanic gardens include conservation collections of rare and themed plant displays, glass houses containing flora from different climatic zones, nurseries, flower display beds, lakes and ponds, and parking for vehicles. Other facilities reported as being

provided by some botanic gardens are visitor information centres, public transport, disabled amenities, venues for hire, children playgrounds, restaurants, picnic facilities, and drinking fountains. In a few cases some botanic gardens have added other facilities such as a library or auditorium for use by the general public. Many studies have found that pleasure and recreational activities (such as improving physical health) are some of the main benefits people seek when visiting a botanic garden so the provision of a variety of facilities significantly improves the overall enjoyment of visitors to botanic garden (Murray et al., 2007; Crilley et al., 2010)

The display of plant collections in different settings is an attractive method to courage people to visit and learn about plant conservation. The exhibition of plants has changed significantly over time as the purpose of the garden has evolved and transformed. For example, in early botanic gardens their main role was viewed as educating the public so plants were commonly displayed in formalised geographic and taxonomic groupings (Oldfield, 2007). Later plant displays became more naturalistic by mixing species with different shapes, colours and textures (Villagra-Islas, 2011). In this context, the survey aimed to recognise how contemporary botanic gardens display plants and whether there are any trends or movements in the way that plant displays are designed and maintained. The survey limited display type to five options though if these were inadequate the respondent was given the opportunity to provide their own description. The majority of respondents stated that their botanic garden displays their plant collections thematically (i.e. as collections consisting of desert species or rainforest species) and geographically; however, they may also utilise other groupings as well such as taxonomic, ecological and horticultural groupings (Figure 4.7).

Engaging and educating the public in conservation issues and informing them about global threats to plant diversity is an essential role of a botanic garden. A question was asked about education activities supported by the responding botanic gardens and the facilities supplied to support this work. The majority of botanic gardens provide guided tours and label their plants and displays using interpretative signage and information leaflets. Some botanic gardens also employ education staff to coordinate and host large numbers of visitors such as children through school visits. Relatively less popular was the use of technology such as educational websites, audio books, computer terminals, and interactive videos. To highlight the importance of education, a recent study found that the majority of conservation research undertaken in botanic gardens is not recognized and remains largely unknown to visitors and the wider public and concluded that there is a

strong need to highlight and better communicate these important functions (Moskwa and Crilley, 2012). Another study on the social role of botanic gardens found that society is unfamiliar with environmental problems such as global warming and identify several areas where botanic gardens can increase awareness about environment issues through 'broadening audiences (audience development), enhancing relevance to communities (meeting the needs of communities), education research which has socio economic impact locally and globally, contributing to public (and political) debates on the environment, modelling sustainable behaviour, actively changing attitudes and behaviour' (Dodd and Jones, 2010).

Another way to engage public is through volunteer programs, which may also provide other benefits from the roles and additional support that they may provide when botanic gardens are understaffed. One of the survey questions asked if the botanic garden engaged volunteers in any capacity and if so what roles and functions they served. The majority (67%) of respondents stated that they have volunteer programs which are commonly utilised for maintenance of the gardens but volunteers were also utilised for running tours, educational programs, and assistance in greenhouses. This result is consistent with another study where volunteers were identified as important through acting as garden guides (42%) and participating in hands-on gardening activities (25%) (Moskwa and Crilley, 2012).

Funding is essential for the smooth operation of a botanic garden as it supports the employment of suitably qualified staff, the maintenance and repair of key infrastructure and the ongoing support and development of conservation and horticultural programs. Consequently, botanic gardens need to identify and secure ongoing funding which may come from different sources so they can operate with a reasonable level of financial security (Olin, 1995). Three questions were asked about sources of funding, the annual budget and how their funds are distributed among the different areas that they support. In terms of their annual budget, the majority of botanic gardens received between $100,000 to $10,000,000 AUD to support their operations with most funding coming from government, though entry fees also contributed significantly to their operating budget. Though less popular, some funding was also secured through sponsorship, donations, universities, consultancy and nursery sales. In most cases, the majority of operational budget was used on staff with 62% of botanic gardens spending 50-75% of their budget employing and supporting staff. It was also found that 81% of botanic gardens spend less than 25% on horticultural displays with only 13% spending considerably more than this

(i.e. 50-75% of their operational budget). Other aspects of their operations, such as conservation, education, and advertising received less than 25% of their annual budget. In comparison, four main sources of funding were identified by Olin (1995) and these were the home unit (or institution), charitable donations, earned income (such as a shop and restaurant) and collaborative efforts such as fund raising and research projects.

Plant collections in botanic gardens represent an important method for *ex situ* conservation. A recent study revealed that botanic gardens around the world manage at least 105,634 species, equating to 30% of all plant diversity and conserve over 41% of all known threatened species (Mounce et al., 2017). Plant collections and their curation represent one of the primary functions of a botanic garden which is even more important when conserving threatened plant species, which in some cases may no longer be found in the wild. For this purpose the survey focussed on quantifying different aspects of the plants collections that respondents were managing to compare and contrast their *ex situ* conservation programs. The majority of botanic gardens (30%) hold 2,000-5,500 plant species with 26% holding less than 600 plant species as part of their botanical collections. In comparison, 11% of respondents have more than 10,000 species in their plant collections. Participants were also asked about the number of accessions held as part of their seedbank, nursery and tissue culture collections (Table 4.5). The results show that most (23) botanic gardens maintain a containerised living collection with the majority (>50%) holding between 1,000-10,000 accessions. It was also found that 21 botanic gardens maintain a seedbank with 28% holding more than 10,000 accessions. Only four of the respondents reported as having a tissue culture collection with the majority (75%) holding less than 10 accessions. The high number of seeds accessions reported as being held in seedbanks by the respondents may be due to lesser space needed and the lower maintenance costs compared to that required for maintaining living plants under nursery conditions. In comparison, tissue culture was less utilised than the other forms of germplasm storage which may be due to its high costs, specialised technical requirements and relative difficulty which will be discussed later in this chapter.

Plant collections can reflect the diversity of plant life from different regions which is either indigenous (native), non-native (exotic) or an artificially bred selection (cultivar) developed under nursery conditions. To determine the original of the plants held by each botanic garden, a question was posed asking where the original plant material was sourced for the plants in their collections. The majority of botanic gardens (91%) hold native flora though for most this accounted for less than 25% of their total plant collection. In

comparison, 83% of botanic gardens hold exotic flora which accounted in the majority of cases for 50-75% of their total plant collections. Various plant cultivars were found in 70% of botanic gardens though these typically accounted for less than 25% of the total number of plants in their collections. Botanic gardens require a comprehensive policy for their plant collections with specific guidelines on their acquisition, content and management. In addition for conservation and restoration purposes, plant collections need to be mainly of wild origin with adequate amounts of genetic diversity and standard quality assurance and take into consideration whether the species has been correctly identified, if it has accurate collection information (i.e. location and date of collection), and whether the plant material is free from plant diseases and potential pests (Heywood, 2010; Blackmore et al., 2011). As an example of potential problems, it was found through a survey of botanic gardens in Europe that most accessions held as part of their collections are being maintained in mixed collections (therefore with a potential for hybridisation) with no sufficient data to identify the collection location and storage history which may negatively impact the use of this material in future reintroduction programs (Maunder et al., 2001).

The main focus of the survey was to develop a deeper understanding of the conservation and research requirements of a botanic garden and how these compare across botanic gardens with quite different structures and functions. The survey revealed that the majority (85%) of botanic gardens consider research and conservation as one of their main functions. As reported by the respondents, the main focus of research were taxonomy (15), ecosystem ecology (14), species recovery (13), horticultural research (13), restoration ecology (12) and seeds conservation (12) covering a broad and diverse group of research disciplines. It was also noticed through the survey that most botanic gardens focussed on conservation research to a lesser or greater capacity. The majority of botanic gardens (74%) reported that they were involved in *ex situ* conservation as part of their conservation research, while less 39% reported that they were undertaking some form of *in situ* conservation. It was also noted that some (9) botanic gardens were utilising both *ex situ* and *in situ* conservation approaches while four botanic gardens reported that they do not engage in either. The greatest proportions of botanic gardens are presently working on the conservation of 10-100 species as part of their overall research programs. While *ex situ* conservation plays an important role in the management of threatened plant species additional studies should also be undertaken to understand the habitat and ecosystem where threatened species occur as this information may be important in future translocation attempts as well as the restoration of degraded habitats which is why Havens et al., (2016) considers it important to coordinate both type of conservation approaches.

One of the intentions of the survey was to highlight any collaborative activities of botanic gardens to illustrate the importance of these types of partnerships for serving plant systematics, conservation, education and public communication. The majority of botanic gardens that responded to the survey stated that they actively collaborate with other botanic gardens, national parks, universities, other miscellaneous organizations and herbaria with a variety of organizations provided as examples of their collaborations such as the BGCI, AMJIS, AIMJB, CETAF, TCI Environmental club, UKOTCF and the Gothenburg Global Biodiversity Centre (Table 4.9). The results confirm that most botanic gardens actively collaborate with other conservation organizations and non-governmental organizations to achieve common conservation goals. However, before the foundation of the BGCI in 1987 it is reported that botanic gardens only rarely collaborated so the founding of the BGCI appears to have significantly improved the capacity of botanic gardens to network and consequently improve the quality and scope of their conservation programs (Blackmore et al., 2011).

Another aspect of the study aimed to understand what botanic gardens considered as a priority for land conservation. The results from this question highlight the differing opinions of land conservation with a diverse range of responses noted. The majority of respondents stated that they viewed ecosystem conservation and native plant conservation as priorities for land conservation. A smaller number considered biodiversity, endangered species and restoration to be the most important factors, while climate change, soil fertility, enhancing traditional management, bioethical principles and seed banks were considered to be priority in the context of the question by only a few of the respondents (Figure 4.11).

In order to understand the types of *ex situ* conservation techniques utilised by different botanic gardens a question was asked about this aspect of their conservation programs. The majority of botanic gardens stated that they use seeds bank (38%) and field gene banks (31%), with DNA banking, *in vitro* storage and pollen banking less popular (Figure 4.15). Propagation and cultivation were selected as the most popular approaches for plant conservation compared to data stewardship which was nominated less frequently. Other suggestions made by respondents included maintaining biological diversity and integrative management of the landscape. The findings from this part of the survey are consistent with another study on European botanic gardens where it was reported that most research is on taxonomy and the display of plant collections (Maunder et al., 2001). Data stewardship is an important aspect in plant conservation, however the survey found that it was the least priority of the botanic gardens and thus may highlight a weakness in conservation

management in some of the botanic gardens. The databases in botanic gardens are meant to support collection, research, conservation and education. The BGCI consider data stewardship an essential aspect in successful conservation and an important tool for information exchange within botanic gardens around the world (BGCI, 2014)

Question 27 asked about the storage duration of seeds within the botanic gardens seedbank. The majority of respondents (35%) stated that their seeds are stored for the medium term (i.e. 25 years) while only 22% stated that they store them for a longer period of time (i.e. 75 years). The standard requirement for storage is that accessions should remain at least 65% viable for 10 to 20 years (FAO/IBPGR, 1994). Interestingly, some botanic gardens stated that they do not operate their own seedbank and store their seeds using facilities supplied by other organisations. Medium to long term storage under standard seed storage conditions may be the most appropriate method for conserving orthodox seeds; however, this approach is not applicable for all species (Martyn *et al.* 2009). Orthodox seeds can usually be stored under standard seedbank conditions by decreasing the moisture content to 3-7% over several weeks then storing the dried seeds at -18°C (Wilhelm, 2004). In comparison, recalcitrant seeded species which may account for around 8% of the world's flora cannot be dried or stored at -18°C because they lose viability so need to be conserved using other approaches (Hawks, 2000; Wyse & Dickie 2016). *In vitro* storage techniques may be one way to store desiccation sensitive taxa, which involves the culturing of seeds/ seed embryos in a pathogen-free environment on agar-based tissue culture media under near perfect growing conditions (Offord et al. 2009). This method is an alternative to field gene banks (i.e. arboretum) which can require considerable space and are prone to pests and diseases and while expensive (in terms of equipment, consumables, facilities and technical support) provides several clear advantages in that much less room is required and the plants are maintained under ideal growing conditions. Nevertheless, there are several potential disadvantages such as a risk of random somaclonal variation and unexpected equipment failure though storing conditioned plant tissues cryogenically in liquid nitrogen that may require improving in the utility of *in vitro* storage approaches (Hamilton et al. 2009; Maxted, 2013).

As many plant species are currently threatened there is increasing pressure on botanic gardens to conserve a greater number of threatened plant species. *Ex situ* conservation through the use of seedbanks and containerised plant collections is considered as a back-up strategy to prevent plant extinction, though in many cases *in situ* conservation is less expensive and potentially more successful (Merritt et al, 2014). Consequently, *ex situ*

conservation should not be used as a replacement for *in situ* measures unless there are no other options (Merritt et al., 2014). The survey tried to identify how botanic gardens assimilate and manage the increasing number of plant species that they have in their collections. The majority manage their collections through maintaining growing plants under nursery and glasshouse conditions or by using seedbanks. Far less commonly utilised at present are advanced technologies such as tissue culture and cryopreservation (Table 4.11).

The final question was posed to gain a better understanding about the activities that botanic gardens currently find challenging due to budget limitations. Judging from the outcomes from this question a significant number of botanic gardens are facing difficulties in terms of being understaffed which was the most common response. In addition to staffing problems, having sufficient space to carry out their work, undertake research or for germplasm storage were also identified as areas where improvements could be made if additional funding was provided (Table 4.12). As an example of where financial constraints and a lack of suitably qualified staff are considered as a significant impediment to conservation is the ecological restoration of Madagascar's 11 priority area by the Missouri botanic garden where a lack of resources has compromised aspects of this program (Birkinshaw et al., 2013). While this limitation has been well described in other studies the results from this survey also suggest a similar story that botanic gardens are generally under resourced with staffing shortages one of the main issues confronting most botanic gardens (Hawks, 2000; Maunder et al, 2001; Maxted, 2013).

The restoration of threatened species and degraded lands is one of the priorities for botanic gardens and for the use of conservation plant collections. The results from this survey show that the majority of botanic gardens (66%) have active restoration programs though these varied considerably across botanic gardens. Botanic gardens are key centres for implementing restoration programs as they house many of the unique skills and resources required for undertaking restoration ecology including the relevant expertise, research and facilities. Nevertheless, on occasion restoration may not work and this restoration failure may be due to a number of reasons such as a lack of biological information on the target species (Hardwick et al., 2011), poor site selection or inappropriate species selection (Wagner et al., 2008). Solutions to these issues may include setting realistic goals, better understanding of the target species and careful consideration of potential constraining factors such as ecological, financial and social aspects related to and underpinning the restoration program (Miller and Hobbs, 2007).

Funding is an important factor for the continuance of any institution including botanic gardens, therefore any shortage in funding affects directly or indirectly their functionality including research and conservation roles. In our study we wanted to identify how a shortage in funding impacts on botanic gardens. The majority of botanic gardens identify being understaffed as a principle reason for funding. As an example, the funding crisis of Israel botanic garden when facing government funding cut that affected directly several aspects in the botanic garden such as salaries, maintenance, garden-wide supplies and as a consequences the botanic garden had to find alternative solutions such as charging for previously free education programs (Owen, 2016).

A possible solution besides securing several funding resources might be to include volunteers and increase their role in botanic gardens. Our results found that about 65% of botanic gardens included volunteers; and that increasing their numbers may help with the lack of staff. An example of the significance of volunteers is at the Kings Park Botanic Garden; The Friends of Kings Park was established in 1993 and volunteers have various roles such as Bushland Carers, Garden Carers, Special Species Growing Group, Administration, Climate watch, Science, Native Plant Sales, and Rio Tinto Naturescape in Kings Park.

Another issue highlighted in this study was the limited places for storage of germplasm. A solution for this may be to use *Ex situ* conservation such as seed banks and *in vitro* storage as only a small area is needed. However, it was found in this study that *in vitro* storage was limited and can be related to funding restriction.

Another concern arising from this study was data stewardship as it was ranked as the least priority for botanic gardens. The ability to conserve correct records about plant species held in institution and being able to share this information is crucial in helping other conservation projects (BGCI, 2016). Botanic gardens need to have a record system to gather their diverse species data and make it available to be searched, filtered, and displayed in a report or exported by other institutions. Another weakness found in this study was the limited number of botanic gardens that undertake *In situ* conservation in comparison to *Ex situ* conservation. *Ex situ* conservation should not be an alternative solution for *In situ* conservation as its goal is to protect endangered plant species in their natural habitat and provide green cover for lands.

Botanical gardens are involved in active plant conservation and several plant species were conserved by Botanic gardens, for example the numbers of case studies in Kings Park

Botanic Garden (KPBG) in Perth/ Western Australia. Scientist in KPBG established numerous and successful conservation projects, for example the threatened species (*Androcalva perlaria*) was discovered recently (1993) with less than 400 plants remaining. A conservation program was established to understand the biology of the species, creating a tissue culture collection, profiling the genetic diversity across populations, assessing the ecophysiology of plants under *in situ* and *ex situ* conditions and developing ways to freeze plant material in liquid nitrogen. In addition this program was able to generate high numbers of seeds (~250,000) for the production of plants and used it to establish new translocated populations around Wellstead (KPBG, 2019). Another example was a program about *Ricinocarpos brevis* species as several field and laboratory experiments were conducted to understand the dormancy alleviation, germination promotion and *in-situ* conditions of natural and recipient translocation site (Turner et al., 2017). Also, Seed Conservation supports the WA Seed Centre - Kings Park which is considered vital for conserving WA's biodiversity through the long-term storage of seeds. Furthermore, these seven study species are part of a project conducted in Kings Park Botanic Garden Restoration Seedbank Initiative project that focusses on four aspects; seed bank management and curation, seed bank science, seed enablement, and development of suitable plant growth media, to deliver the capacity to restore landscapes following mining using seed-based technologies.

Chapter 5 General discussion

5.1 Introduction

This chapter consolidates the two broad themes covered, namely investigating the germination of selected seeds from arid regions for restoration and land conservation purposes and the role of botanic gardens as related to plant conservation, which collectively covers the three research chapters. It is well recognised that seeds are an important element for conservation and restoration ecology with an improved knowledge on their traits being essential to increase their effectiveness in vegetation re-establishment in restoration programs. This study has subsequently improved the knowledge of seed germination of arid plant species which will further improve the success of revegetation and conservation programmes in these regions.

The seeds of selected plant species from the semi-arid Pilbara region in Western Australia were firstly studied for a range of traits and classified them into either non-dormant or dormant seeds (Chapter 2). For those species with dormant seeds, the class of dormancy was then defined and a range of experiments undertaken to find practical methods to break dormancy by using several different procedures previously demonstrated to be effective (Chapter 2). For those species found to have non-dormant seeds a novel technique (hydropriming) for enhancing the germination of seeds was investigated (Chapter 3). Seeds from selected species were primed for several periods of time, dried and then tested for germination under different water potentials to simulate the effects of mild to severe water stress on germination (Chapter 3). Finally, to improve our understanding of the various roles that botanic gardens serve from different parts of the world and how these aid conservation and restoration of lands in general, a survey of botanic gardens was conducted (Chapter 4). This information enabled key information about conservation and restoration programs undertaken by different botanic gardens, the size of their collections, workforce and budgets as well as the types of extension and education programs in which they are actively engaged to be determined.

5.2 Seed biology in relation to restoration in a semi-arid environment

The Pilbara region contains a wealth of biodiversity including ~1,800 plant species from common families such as the Fabaceae, Poaceae, Malvaceae, Asteraceae, Cyperaceae and Goodeniaceae (Erickson et al., 2016b). Seeds are a challenging factor in restoration programs as they may not germinate for many reasons including empty florets, viability, dormancy or suboptimal germination conditions (Erickson et al. 2016). The study examined several species representative of some of the most dominant understory families in the Pilbara namely the Poaceae, Cyperaceae and Goodeniaceae as a means to understand their germination traits as well as to determine whether the seeds of any/all of these species possess dormancy and if so what kind of dormancy they may have. Baskin and Baskin (2004a) classify seed dormancy into five main classes based on various seed attributes, the dormancy mechanism and the methods required to break it. Several analyses were used to define whether seeds from the study species exhibit dormancy through understanding a range of seed characteristics. In addition to this, seed viability was also investigated as this is a common problem with the seeds of many Australian native species (Merritt et al. 2007; Martyn et al. 2009). Seed attributes investigated during this study included:

1. Determining embryo type through dissecting seeds;
2. Analysis of seed fill by using an X-ray machine;
3. A cut test and tetrazolium test to determine if filled seeds were dead or damaged (i.e. were viable);
4. Determination of seed weight; and
5. Embryo:Seed ratio to provide evidence as to whether the embryo is small relative to the size of the seed.

Seeds fill ranged between 86 - 100% across all species, so clearly seed fill was high with the species studied. In comparison, seed viability was also high and ranged between 75 - 96% for the three *Goodenia* species, therefore it was not assessed. Seed weight ranged between 0.15 - 1.64 mg/seed and E:S ratio ranged between 0.33 - 0.66 mm, so all the species investigated in this study possessed endospermous seeds. As well, seed features and class of dormancy were also defined with all species exhibiting fully developed embryos with three different types of embryos observed, namely; lateral, capitate and spatulate, strongly suggesting that none of these species possessed ether morphological or morphophysiological dormancy as per Baskin and Baskin (2004a). For defining if seeds exhibited physical

dormancy, permeability of the seed coat to water was determined, which indicated that all seeds imbibed water readily and none came from a family previously found to contain species outlined by Baskin and Baskin (2004a) having physically dormant seeds. These results strongly indicated that seeds did not have physical dormancy, the second most common form of dormancy found in arid lands. It was found that four species (*Cymbopogon obtectus*, *Eriachne mucronata*, *Goodenia armitiana* and *Goodenia cusackiana*) had non-dormant seeds and three species contained seeds with physiological dormancy (*Eragrostis eriopoda*, *Fimbristylis dichotoma* and *Goodenia stobbsiana*). Seeds of *Cymbopogon obtectus* and *Eriachne mucronata* were found to have high to very high germination without their florets, though *Cymbopogon obtectus* also had high germination even when the florets were still present surrounding the seeds. Interestingly, the germination of *Eriachne mucronata* seeds decreased when germination was compared to seeds with florets, which may illustrate that seeds require cleaning before their use in restoration programs and that the surrounding floret acts as a mechanical barrier that restricts germination to some degree.

The response to temperature varied with different species and there was no one temperature that suited all seven species. For *Eragrostis eriopoda* and *Fimbristylis dichotoma* it was difficult to initially define the temperatures most appropriate for their germination; after several experiments using 20, 25, 30, and 35°C no germination was observed and there was no clear understanding as to why this was the case. To explore whether it was a germination problem (i.e. inappropriate temperature regime) or a seed dormancy block germination was tested at a higher range of constant and alternating temperatures (i.e. 20/35, 40, 25/40, 25/45, 50, and 25/50°C). Test germination was conducted under different temperatures and media types containing different germination stimulants such as GA3 and KAR1. The assessment of additional temperatures proved quite helpful as several treatment combinations (around 40°C) resulted in some germination; however, germination still did not exceed 5% in *Eragrostis eriopoda* suggesting that the seeds of this species have deep physiological dormancy. Different germination media were also ineffective in improving *Eragrostis eriopoda* germination, though for *Fimbristylis dichotoma* incubating seeds on KAR_1 was found to be an effective way to increase germination when these were incubated at 25/40°C. In comparison, germination of *Cymbopogon obtectus* and *Eriachne mucronata* seeds were found to be both high and similar across a range of cooler temperatures (20, 25, 30, 35°C) highlighting the variability in response to germination temperature.

Goodenia species were also found to germinate under a wide range of constant and alternating temperatures though they displayed quite different responses to the different germination stimulants assessed. For the seeds of *Goodenia stobbsiana,* germination was highly influenced by the use of the GA_3 incubation medium as it improved germination in most temperatures compared to either water or KAR_1. The exception to this general trend was observed for seeds incubated at 25°C where the effects of KAR_1 and GA_3 were generally quite similar. For *Goodenia armitiana* and *Goodenia cusackiana* temperature proved to have more of an impact on germination than the type of incubation media (i.e. water, GA_3 or KAR_1 with both 25 and 20/35°C found to be preferable for germination while the type of incubation media had a similar effect for both species. These results are important to establish what is helpful or not to increase germination and will contribute to successful restoration outcomes for this species.

This study highlighted the importance of examining seeds before using them in plant conservation and restoration ecology as they may not germinate for many reasons including empty florets, viability, dormancy or suboptimal germination conditions, for example the temperature requirements were different for each species and some species exceed what was expected with the temperature requirements.

5.3 Overcoming seed dormancy as an essential treatment prior to restoration

Physiological dormancy was found to inhibit the germination of several semi-arid species from the Pilbara region. In order to break dormancy and enhance germination three methods were investigated that were previously found to release physiological dormancy in other Australian semi-arid taxa including species of Goodeniaceae and Poaceae (Hoyle et al. 2008; Erickson et al. 2016). These approaches included dry after ripening, wet/dry cycling and seed coat scarification (Hoyle et al., 2008a; Hoyle et al., 2008b; Commander et al., 2009; Farley et al., 2013; Erickson et al., 2013a). For afterripening treatments, several temperatures were examined for different time periods with the most successful afterripening temperature found for the seeds of *Eragrostis eriopoda* being 50°C applied for 26 weeks. Combining these afterripening conditions with the incubation of seeds at 25/45°C further improved germination for this species. In comparison, afterripening was found to be ineffective in increasing germination in *Fimbristylis dichotoma* seeds, with several afterripening treatment combinations

actually lowering germination. However, the use of wet/dry cycling at 30°C for 18 months significantly increased germination when seeds were incubated at 40°C following the wet/dry cycling treatment. The application of afterripening and wet/dry cycling temperatures to break dormancy in combination with high incubation temperatures (~40°C) to assess the germination responses were both essential for enhancing germination in these two species. Although both species are from the Pilbara region, the requirement for KAR_1 was different with the seeds of *F. dichotoma* showing more of a KAR_1 response on occasion though this was not particularly uniform or indeed very high.

All three *Goodenia* species responded better to cooler incubation temperatures (25°C or 20/35°C) with each showing some improvements in germination response to the use of KAR_1 when seeds were exposed to afterripening conditions for different lengths of time. However, afterripening was only found to be partially effective in some treatment combinations (not all) which was more noticeable in the seeds of *G. armitiana* and *G. stobbsiana.* In comparison the seeds of all three *Goodenia* species responded poorly to wet/dry cycling with a general reduction in germination noted as seeds were exposed to this treatment over time. Overall, due to physiological dormancy in *Goodenia stobbsiana* seeds the highest germination never exceeded 65% while in *Goodenia armitiana* and *Goodenia cusackiana* seeds it reached >95% as they were not dormant and their initial germination was high.

Acid scarification of *Eragrostis eriopoda* seeds improved germination compared to non-treated seeds; however, across all the treatment combinations assessed germination never exceeded 20%. Similarly, acid scarification also improved germination in *Fimbristylis dichotoma* seeds though germination did not exceed 50%. Given that there was some improvement in germination (albeit quite a modest one) it can be inferred that in these two species the seed coat plays an important role in restricting embryo growth in some way and therefore reducing germination. While mechanical scarification was found to be largely ineffective in both species resulting in poor germination, it may not be directly related to the effectiveness of the scarification treatment itself but more likely to the small seed size of both species and likely to have impacted the effectiveness of the scarification process.

The study identified pre-treatments that can be used for the seven species when using in conservation programs. Our results show that it is recommended for the seeds of *Eragrostis eriopoda* to use afterripening at 50°C applied for 26 weeks. Combining these afterripening conditions with the incubation of seeds at 25/45°C further improved

germination for this species; however, further research should be done to achieve for a higher germination. Afterripening was found to not be useful for *Fimbristylis dichotoma* seeds but the use of wet/dry cycling at 30°C for 18 months significantly increased germination when seeds were incubated at 40°C following wet/dry cycling. Scarification was another method that proved to have some results for previous species, however should be furthered studied. For the *Goodenia* species the pre-treatment requirements are cooler temperature (25°C or 20/35°C) with the use of KAR1 in afterripening; however, all *Goodenia* species did not respond effectively to a wet/dry cycle.

5.4 Enhancement of seed germination in arid regions

Priming simulates rapid wetting and dry cycles that happen naturally in the soil as seeds persist in the soil seed bank, such as the climate experienced in the semi-arid tropical Pilbara region with its sporadic rainfall and high evaporation during summer. Priming is a cost efficient and environmentally friendly method to enhance germination, decrease germination time as well as to enhance the ability of germinating seeds to tolerate moisture limiting conditions. The study analysed the effects of priming by using different priming times (3 h, 6 h, and 9 h) and then examining the germination capacity of primed seeds after incubating on a range of water potentials (0, -0.25, -0.5, -1.0 MPa) simulating nil to severe moisture stress. Results indicate that the seeds of *Cymbopogon obtectus* are more sensitive to drought stress than *Eriachne mucronata* as even though priming for 6 h improved germination under medium water stress (-0.5 MPa) germination decreased under severe drought stress (-1.0 MPa) indicating that priming is ineffective at this point. Interestingly, for the seeds of *Eriachne mucronata,* germination was not affected by extreme water stress (-1.0 MPa) so seeds did not show any improvements in their capacity to tolerate moisture limiting conditions. However, priming did improve the germination rate for both species which may still be helpful for restoration programs in arid lands where water is limited.

It was found through the study that priming techniques are helpful in increasing germination parameters under sever conditions as per arid land, thus it is recommended to use this technique in the studied species *Cymbopogon obtectus* and *Eriachne mucronata* for restoration programs in arid lands.

5.5 The structure of Botanic gardens as related to plant conservation

Botanic gardens are institutions that have high potential to contribute to plant conservation and land restoration through devoting expertise, facilities and space to the pursuit of these important objectives. Botanic gardens over time have served several roles; however, the importance of conservation has risen significantly in recent times with the increasing risk of biodiversity loss and plant extinction. Botanic gardens have proven their efficiency and effectiveness in several conservation areas around the world (O'Donnell and Sharrock, 2017) although conservation is very limited in arid land regions despite the fact that this area contain one- fifth of the world species being one the most threatened ecosystems globally (van Slageren, 2003). This study gathered information from botanic gardens from different continents and climates around the world to better understand how botanic gardens manage their resources to serve a conservation agenda and to define and understand where limitations exist. Despite the limited number of participants (27) to the survey, the responses were both valuable and insightful in increasing our knowledge and analysing trends in the majority of botanic gardens. The survey also proved useful in detecting some of the challenges currently facing botanic gardens and reflecting their status and function as it gave a broad view on their structure, plant collections, financial conditions, public education and extension programs, volunteer roles, research activities, collaboration, conservation and restoration programs.

The majority of botanic gardens considered plant display, education, conservation and research their first priority regardless of their location or size. Engaging the public is important to support conservation aspects and increase knowledge on global threats. Botanic gardens have better opportunity to both interpret and convey these messages to large communities with many visitors attending annually as highlighted through the survey with most botanic gardens open to the public. In addition, they also actively engage with volunteers through various volunteer programs to increase peoples' awareness and interaction with botanic gardens and some volunteers also assisting in the upkeep and maintenance of botanic gardens filling important and indeed critical roles that keep the garden functioning on a daily basis. Botanic gardens also offer many different facilities to serve a wide range of visitors to make their experience as valuable as possible; a common outcome from botanic gardens surveyed. Another method to engage the public and increase their knowledge on plants is through education facilities. It was illustrated through the survey that the majority of botanic gardens included plant labelling, guided

tours and informative leaflets, while a minority also included technology as part of their education and extension programs. One theme the survey focused on was the display of plant collections in botanic gardens which are essential for encouraging people to visit and it was noted that plants are most often displayed thematically and geographically for groups of different plant species.

The survey highlighted financial issues as a critical aspect for botanic gardens to manage and constantly adapt to but in many situations is was not well addressed or even understood (Olin, 1995). The majority of botanic gardens were dependent mainly on government funding and to a lesser degree on entry fees whereas a minority were also reliant on sponsorship, donations, universities, consultancy fees and nursery sales to provide money for their ongoing operations and management. The majority received between 100,000 to 10,000,000 $AUD to support their operation while most of the funding was dedicated to staff with the minority (less than 25%) of their budget spent on horticultural displays, conservation and education.

The majority of botanic gardens (8) held 2,000-5,500 plant species with seven found to hold less than 600 plant species, which indicated the range of capacities that botanic gardens possess to include plant species in their plant collections. Accession numbers also varied between botanic gardens with the majority of accessions being held either in nurseries (a majority (7) hold 1,000-10,000 accessions) or in seeds banks (majority (6) hold more than 10,000 accessions). By comparison far fewer accessions were being held in tissue culture collections (majority (3) hold less than 10) with 11 botanic gardens found not to have any tissue culture accessions and may indicate the limited usage of tissue culture in botanic gardens. Living species in the majority of botanic gardens were mostly natives with fewer species being exotic and still fewer being cultivars.

The survey illustrated that the majority of botanic gardens considered research and conservation as one of their principle functions with the greatest research focus being taxonomy, ecosystem ecology, species recovery, horticultural research, restoration ecology, and seed conservation. The highest proportion of botanic gardens stated that between 10-100 species formed part of their current research programs. From previous research, conservation was also identified as one of the main functions of botanic gardens and while the majority of botanic gardens were involved with some aspect of *ex-situ* conservation far less reported undertaking *in-situ* conservation activities. The main *ex- situ* technique used by botanic gardens was seed banking with a large number also reporting the use of field gene banks. Far less popular was DNA banking, *in vitro* storage and pollen

banking. Most botanic gardens used a seed bank plan to store their seeds for the medium term while several botanic gardens stored their seeds in the facilities of other organizations as an alternative to having their own seed bank. The aim of most botanic gardens working on plant conservation projects was propagation and cultivation of the target species, while of secondary importance was capturing a broad range of genetic diversity and data stewardship. With the increasing number of *ex-situ* conservation programs the survey identified that most botanic gardens managed the storage capacity of their *ex situ* collections via nurseries and seed banks and far less by cryopreservation and tissue culture. Most botanic gardens were involved in restoration programs to some degree; however, most also dedicated a large proportion of this activity to the restoration of the botanic garden itself.

One essential feature of botanic gardens was collaboration activities and the survey tried to highlight this and inquired about type of partnerships that different botanic gardens may be involved with. The majority of participants collaborated with other botanic gardens (through the BGCI for example), national parks, universities, herbaria and various NGOs which may improve the effectiveness of botanic gardens in future, particularly as they work toward specific conservation targets. Priorities in conservation was one of the queries that was thought to be useful to understand from a botanic gardens perspective, thus the reason for the open-ended question in order to capture a range of different views. The majority of respondents stated that they considered ecosystem conservation and native plant conservation as their highest priorities though other views were also suggested. The major challenges that botanic gardens faced was due to limited budgets with most botanic gardens stating that they have a shortage of staff, lacked sufficient research facilities and were constrained by space limitations such as germplasm storage areas.

Botanic gardens as we largely know them have been around for several centuries though their roots are much older. Throughout history botanic gardens have been critical to the study of plants and the collection and maintenance of irreplaceable collections of economically, medicinally, horticulturally and agriculturally important plants that have at times changed the course of history and led to both the building of empires as well as their downfall. More recently however, botanic gardens have now found themselves at the forefront of plant conservation and house important stores of critically endangered species that in some cases are no longer found in the wild. A large part of these conservation collections are maintained as seeds in seed banks which in most cases are quite poorly understood and in many instances cannot be reliably germinated due to seed dormancy or a lack of knowledge about

their germination requirements. If botanic gardens and the seeds that they contain are going to be effective tools for undertaking ecological restoration in future then more must be done to understand the seeds in these banks and how best these can be effectively utilised. This study has attempted to address this knowledge gap through documenting key seed attributes, understanding the germination requirements, determining the most effective way to break dormancy and by improving the germination capacity of non-dormant seeds via seed priming. All this seed-focussed work was undertaken in a range of wild species currently needed for land restoration with this knowledge then placed within the context of the modern botanic garden whose role is increasingly being modified and reshaped in a rapidly changing world.

The wide range of information collected has enabled a comprehensive understanding of botanic gardens to be obtained and expose any challenges that might appear avoidable in the future. The main concern arising from this study being data stewardship as it was assigned the lowest priority. Botanic gardens may play a bigger role in global conservation if data stewardship is set as a higher priority. Another concern was the limited use of *In situ* conservation, thus botanic gardens should increase their effort in undertaking *In situ* conservation as it will directly affect positively endangered plant species in their natural habitat and provide green cover for lands mainly for arid land conservation. Other challenges included funding which could be solved by securing several funding resources and increasing the role of volunteers in botanic gardens. Limitation of places for storage was another challenge which could be solved by researching alternative storage methods for different plant species. Overall the study highlighted the strong connection between botanic gardens and the conservation of plant species and identified difficulties they faced in able to find alternative solutions.

5.6 Final conclusions

Overall, the key findings of this study were as follows:

• Seeds from different species from the same region require quite different temperatures for germination. For example, high temperatures (>40°C) were required for germination of *Eragrostis eriopoda* and *Fimbristylis dichotoma* seeds, which also had quite specific temperature requirements. In comparison, the seeds of the Goodenia species germinated over a broad range of temperatures, while those of *Cymbopogon obtectus* and *Eriachne mucronata* had the highest (>80%) germination over a large range of temperatures (20, 25, 30 and 35°C).

- Seeds exhibit different response to germination stimulants; *Cymbopogon obtectus, Eragrostis eriopoda, Eriachne mucronata* and *Goodenia armitiana* did not respond to any stimulant, while *Fimbristylis dichotoma* and *Goodenia cusackiana* had higher germination in KAR1, whereas *Goodenia stobbsiana* responded better to treatment with GA3.

- Removing the surrounding florets from *Eriachne mucronata* seeds significantly improved germination (>90%), yet there was no requirement for removing the surrounding florets from *Cymbopogon obtectus* seeds as germination was very high (>90%) for both florets and seeds.

- Physiological dormancy was identified in three species (*Eragrostis eriopoda, Fimbristylis dichotoma* and *Goodenia stobbsiana*) that required different treatments for it to be overcome. Dormancy in *Eragrostis eriopoda* seeds was partially released using an afterripening treatment under high temperature (50°C) whereas dormancy in *Fimbristylis dichotoma* seeds was partially released through wet/dry cycling. *Goodenia stobbsiana* seeds were also partially released from dormancy through dry afterripening at warm temperatures.

- Scarification with sulfuric acid was useful to a small extent for overcoming dormancy in *Eragrostis eriopoda* and *Fimbristylis dichotoma* seeds; however, mechanical scarification was ineffective in breaking dormancy in both species.

- Priming non-dormant seeds of *Cymbopogon obtectus* and *Eriachne mucronata* was useful in enhancing germination under some water limiting conditions as well as decreasing the germination time (t10 & t50).

- Botanic gardens contributed to the conservation of plant species and participated in restoration programs through the provision of unique technical facilities and expertise that may not be found in other institutions. The survey highlighted the focus of botanic gardens towards conservation goals, how they managed their resources and pursued this mission with the main obstacles currently faced being lack of funding, expertise and limitation of space and research facilities.

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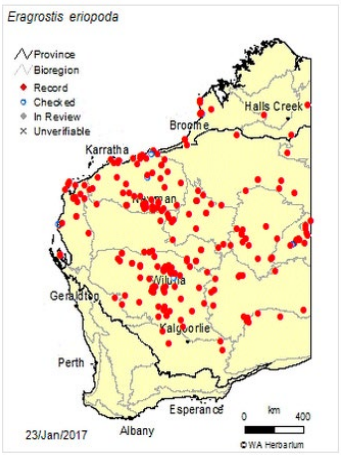
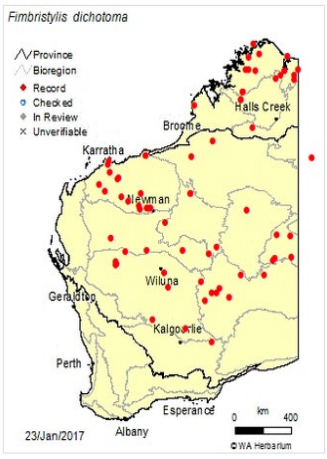
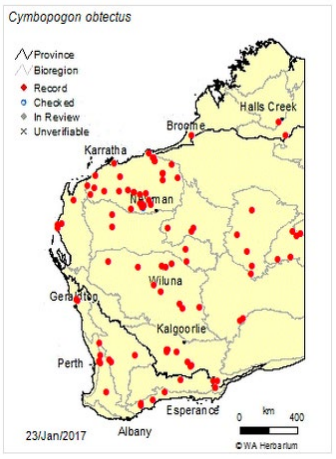
APPENDICES

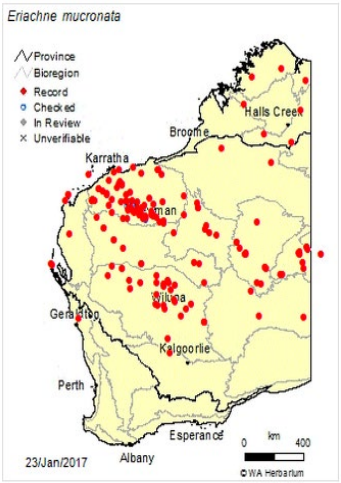
Appendix A Distribution maps of the 7 species in this study

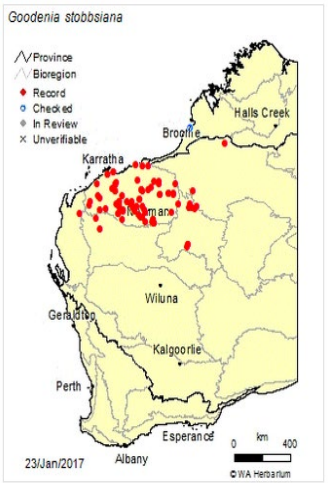
(Western Australia herbarium, 1998)

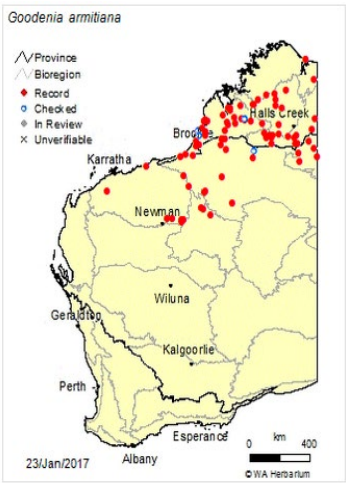


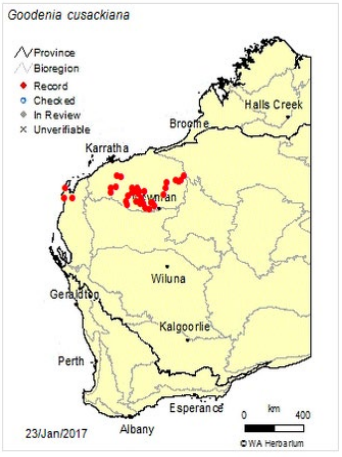












Appendix B Letters of approval

B.1 Ethics approval

Curtin University

Office of Research and Development

GPO Box U1987
Perth Western Australia 6845

Telephone +61 8 9266 7863
Facsimile +61 8 9266 3793
Web research.curtin.edu.au

16-Oct-2017

Name: Deborah Pritchard
Department/School: Department of Environment and Agriculture
Email: D.Pritchard@curtin.edu.au

Dear Deborah Pritchard

RE: Ethics Office approval
Approval number: HRE2017-0735

Thank you for submitting your application to the Human Research Ethics Office for the project **Identify the structure of botanical gardens that enable a more functional design that focus on phytogeographic regions**.

Your application was reviewed through the Curtin University Negligible risk review process.

The review outcome is: **Approved**.

Your proposal meets the requirements described in the National Health and Medical Research Council's (NHMRC) *National Statement on Ethical Conduct in Human Research (2007).*

Approval is granted for a period of one year from **16-Oct-2017** to **15-Oct-2018**. Continuation of approval will be granted on an annual basis following submission of an annual report.

Personnel authorised to work on this project:

Name	Role
Pritchard, Deborah	CI
Nayyef, Alaa Shallal	Student

Approved documents:

Document

Standard conditions of approval

1. Research must be conducted according to the approved proposal
2. Report in a timely manner anything that might warrant review of ethical approval of the project including:
   - proposed changes to the approved proposal or conduct of the study

- unanticipated problems that might affect continued ethical acceptability of the project
- major deviations from the approved proposal and/or regulatory guidelines
- serious adverse events

3. Amendments to the proposal must be approved by the Human Research Ethics Office before they are implemented (except where an amendment is undertaken to eliminate an immediate risk to participants)
4. An annual progress report must be submitted to the Human Research Ethics Office on or before the anniversary of approval and a completion report submitted on completion of the project
5. Personnel working on this project must be adequately qualified by education, training and experience for their role, or supervised
6. Personnel must disclose any actual or potential conflicts of interest, including any financial or other interest or affiliation, that bears on this project
7. Changes to personnel working on this project must be reported to the Human Research Ethics Office
8. Data and primary materials must be retained and stored in accordance with the Western Australian University Sector Disposal Authority (WAUSDA) and the Curtin University Research Data and Primary Materials policy
9. Where practicable, results of the research should be made available to the research participants in a timely and clear manner
10. Unless prohibited by contractual obligations, results of the research should be disseminated in a manner that will allow public scrutiny; the Human Research Ethics Office must be informed of any constraints on publication
11. Approval is dependent upon ongoing compliance of the research with the Australian Code for the Responsible Conduct of Research, the National Statement on Ethical Conduct in Human Research, applicable legal requirements, and with Curtin University policies, procedures and governance requirements
12. The Human Research Ethics Office may conduct audits on a portion of approved projects.

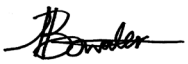
Special Conditions of Approval

Recruitment material - please include the 1st sentence of the HREC statement - *Curtin University Human Research Ethics Committee (HREC) has approved this study (HREC number XX/XXXX)*

This letter constitutes low risk/negligible risk approval only. This project may not proceed until you have met all of the Curtin University research governance requirements.

Should you have any queries regarding consideration of your project, please contact the Ethics Support Officer for your faculty or the Ethics Office at hrec@curtin.edu.au or on 9266 2784.

Yours sincerely



Amy Bowater
Acting Manager, Research Integrity

B.2 Amendment request approval

Curtin University

Office of Research and Development

GPO Box U1987
Perth Western Australia 6845

Telephone +61 8 9266 7863
Facsimile +61 8 9266 3793
Web research.curtin.edu.au

24-Nov-2017

Name: Deborah Pritchard
Department/School: Department of Environment and Agriculture
Email: D.Pritchard@curtin.edu.au

Dear Deborah Pritchard

RE: Amendment approval
Approval number: HRE2017-0735

Thank you for submitting an amendment request to the Human Research Ethics Office for the project **Identify the structure of botanical gardens that enable a more functional design that focus on phytogeographic regions**.

Your amendment request has been reviewed and the review outcome is: **Approved**

The amendment approval number is HRE2017-0735-02 approved on 24-Nov-2017.

The following amendments were approved:
Inclusion of additional question in the survey asking participants for the name of their botanic garden. Participants will be informed that it is their choice whether to answer this question or not, and that the information they provide in this question will only be used to update the BGCI GardenSearch record, and it will not be used in the research which the researchers are undertaking.

Any special conditions noted in the original approval letter still apply.

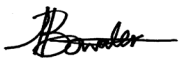
Standard conditions of approval

1. Research must be conducted according to the approved proposal
2. Report in a timely manner anything that might warrant review of ethical approval of the project including:
   - proposed changes to the approved proposal or conduct of the study
   - unanticipated problems that might affect continued ethical acceptability of the project
   - major deviations from the approved proposal and/or regulatory guidelines
   - serious adverse events
3. Amendments to the proposal must be approved by the Human Research Ethics Office before they are implemented (except where an amendment is undertaken to eliminate an immediate risk to participants)
4. An annual progress report must be submitted to the Human Research Ethics Office on or before the anniversary of approval and a completion report submitted on completion of the project
5. Personnel working on this project must be adequately qualified by education, training and experience for their role, or supervised
6. Personnel must disclose any actual or potential conflicts of interest, including any financial or other interest or affiliation, that bears on this project

7. Changes to personnel working on this project must be reported to the Human Research Ethics Office
8. Data and primary materials must be retained and stored in accordance with the Western Australian University Sector Disposal Authority (WAUSDA) and the Curtin University Research Data and Primary Materials policy
9. Where practicable, results of the research should be made available to the research participants in a timely and clear manner
10. Unless prohibited by contractual obligations, results of the research should be disseminated in a manner that will allow public scrutiny; the Human Research Ethics Office must be informed of any constraints on publication
11. Ethics approval is dependent upon ongoing compliance of the research with the Australian Code for the Responsible Conduct of Research, the National Statement on Ethical Conduct in Human Research, applicable legal requirements, and with Curtin University policies, procedures and governance requirements
12. The Human Research Ethics Office may conduct audits on a portion of approved projects.

Should you have any queries regarding consideration of your project, please contact the Ethics Support Officer for your faculty or the Ethics Office at hrec@curtin.edu.au or on 9266 2784.

Yours sincerely



Amy Bowater
Acting Manager, Research Integrity