Germination of *Acacia harpophylla* (Brigalow) seeds in relation to soil water potential: Implications for rehabilitation of a threatened ecosystem

Initial soil water conditions play a critical role when seeding is the primary approach of revegetation on post-mining areas. In some semi-arid climates, such as the Brigalow Belt Bioregion in eastern Australia, extensive areas are affected by surface mine developments. Together with erratic rainfall patterns and clayey soils water deficit is the primary environmental stressor, which makes the Brigalow Belt representative for other water-limited ecosystems worldwide. Apart from other environmental stressors germination is governed by the water potential of the surrounding soil material. While previous studies confirmed the high tolerance of Brigalow (*Acacia harpophylla*) seeds to a broad range of temperature and salinity, the question remains how soil water potential triggers seed germination. In this study, we used three replicates of 50 seeds of predominant native Brigalow to investigate germination in relation to water potential as environmental stressor. Solutions of Polyethylene Glycol (PEG 6000) were applied to expose seeds to nine osmotic water potentials ranging from soil water saturation (0 kPa) and field capacity (-10 to -30 kPa) to the permanent wilting point (-1,500 kPa). We measured germinability (number of germinated seeds relative to total number of seeds per lot) and mean germination time (mean time required for maximum germination of a seed lot) to quantify germination. Based on these empirical data we estimated the parameters of the hydrot ime model, which facilitates to simulate timing and success of seed emergence. Our findings indicate that Brigalow seeds are remarkably water stress tolerant with germination being observed at a water potential as low as -1,500 kPa. Likewise, the average base water potential of a seed population (hydrot ime model) was very low and ranged between -1,533 kPa and -1,451 kPa. In general, Brigalow seeds germinate opportunistically over a broad range of abiotic conditions related to temperature, salinity, and water availability. Direct seeding and germination of native plants on post-mining land may be an effective and economically viable solution in order to re-establish plant communities.
However, due to their ability of asexual reproduction, alternative rehabilitation approaches such as transplantation of whole soil-root compartments may become attractive for restoration ecologists to achieve safe, stable, and non-polluting ecosystems.
1 Sven Arnold1*, Yolana Kailichova1, Thomas Baumgartl1

2 1 Centre for Mined Land Rehabilitation, The University of Queensland, Brisbane, Australia

3 Correspondence: Sven Arnold (s.arnold@uq.edu.au)
1 Introduction

The Brigalow Belt is an extensive bioregion located between the subtropical coastline and semi-arid interior of eastern Australia (Arnold et al. 2013). Climatically and pedologically the bioregion is distinct from surrounding areas (Isbell 2002; Lloyd 1984), and as a consequence, ecological and biodiversity attributes are very unique in Australia and worldwide (Johnson 1980). Rainfall events occur erratically, both between years and intra-seasonally, and the clayey soils have high fertility and good water holding capacities (Gunn 1984). Only a few plant species are able to proliferate under these water-limited constraints, of which *A. harpophylla* (Brigalow) is the predominant species, and from which the bioregion derives its name.

Since the 1950s, the delicate balance between soils and the native plant communities has been disrupted (Evre et al. 2009) through clearance of Brigalow woodland for agricultural land use (cropping, grazing), which reduced the original extension of the bioregion dramatically (Arnold et al. 2013). Consequently, the bioregion has been listed as endangered under both state (Vegetation Management Act 1999) and national legislation (Environment Protection and Biodiversity Conservation Act 1999: EPBC Act) (DEWHA 2010). More recently, areas in the Bowen Basin (largest coal reserve in Australia) have been concurrently affected by surface mine developments. The legislative requirement to reclaim post-mining land to provide safe, stable, and non-polluting environments (Australian Government 2006) provides the opportunity to re-establish native Brigalow plant communities.

Re-vegetation typically comprises either passive regeneration or proactive management such as transplanting seedlings and mature stands (Musselman et al. 2012), tube stocking, or direct seeding. The latter may be an effective and economically viable solution in order to re-establish Brigalow plant communities (Engel & Parrotta 2001; Lamb et al. 2005; Reichman et al. 2006). In this regard, seed germination is an important indicator of re-vegetation success as it represents the first stage of plant physiological response and development to environmental conditions. Seed germination can be controlled by a range of abiotic stressors of which temperature and salinity have only limited impact on *A. harpophylla* seeds within the ranges of 15 to 38°C and 0 to 20 dS/m, respectively (Reichman et al. 2006).

However, given the unique pedological and climatic conditions of the Brigalow Belt, the question remains how Brigalow seeds respond to changes in water availability from soil. This quantitative information is crucial to simulate timing and success of seed emergence, for example when applying the hydrotime model (Bradford 1990; Gummerson 1986).

Although being the major environmental stressor in the Brigalow Belt Bioregion, less information exists on the direct effect of soil water potential on germination of *A. harpophylla* seeds. Therefore, the primary aim of this study is to determine their germination response in relation to soil water potential. As a secondary goal and based on the empirical data of the germination trials we estimate the parameters of the hydrotime model for *A. harpophylla*. Finally, we discuss our findings in the context of previous seed germination trials and alternative opportunities to re-establish plants on post-mining areas under water limited conditions.
2 Materials and Methods

2.1 Experimental design

We used seeds of *A. harpophylla* to investigate germination in relation to water potential as environmental factor. Three replicates of 50 seeds per treatment (i.e., water potential) were placed at equal distance on an absorbent substrate (Wettex®). Materials (e.g., tweezers, Wettex®, glassware) were autoclaved for 20 minutes at 121°C and preparation of treatments as well as any monitoring took place in a laminar flow cabinet. All treatments and replicates were then placed randomly within a germination cabinet under constant temperature (25°C) and a 12 hour day and night cycle. Seeds were removed from petri dishes once a perceptible radicle emerged. The experiment ceased after five days with no further germination. No pre-treatment of seeds was required for the selected species to break dormancy (Schmidt 2000; Turnbull & Martensz 1982).

For controlled experiments on seed-soil relations the osmotic water potential can be used to represent soil matric potential (Mcwilliam & Phillips 1971). We used solutions of Polyethylene Glycol (PEG 6000) to expose seeds to nine osmotic water potentials: 0, -10, -30, -100, -250, -500, -750, -1000, and -1500 kPa. These values capture soil water conditions ranging from water saturation (0 kPa) and field capacity (-10 to -30 kPa) to the permanent wilting point (-1,500 kPa). The empirical equation derived by Michel & Kaufmann (1973) and revised by (Wood et al. 1993) was used to set up the required water potential ($\psi$ in kPa):

$$\psi = \left(6.3 \times 10^{-5} T - 0.02196\right) O^{2.3577},$$

where $T$ is the temperature in K (here 298.15 K), and $O$ denotes the osmolality in g 1,000 g$^{-1}$ of water. Solutions measuring 15 ml of PEG 6000 were added to the seeds on the Wettex® substrate within a 90 mm petri dish and wrapped with Parafilm® to prevent evaporation.

2.2 Germinability and mean germination time

We used germinability $G$ (%) and mean germination time $\bar{t}$ (days) as measurements to quantify germination (Ranal & Santana 2006). While $G$ simply represents the number of germinated seeds ($g$) relative to the total number of seeds per replicate ($n$):

$$G = \frac{g}{n} \times 100,$$

$\bar{t}$ denotes the mean length of time required for maximum germination of a seed lot (Czabator 1962):

$$\bar{t} = \frac{\sum_{i=1}^{k} g_i t_i}{\sum_{i=1}^{k} g_i},$$

where $t_i$ is the time elapsed from initiation of the experiment to the $i^{th}$ observation day until germination ceases on the $k^{th}$ day.

We applied a generalised logistic regression model (GLM) on a logit scale and simple linear regression for $G$ and $\bar{t}$, respectively, to test the significance of the relationship between germinated seeds or time required for maximum germination and decreasing water potential. Assumptions were tested...
via diagnostic plots, which showed no violation of homogeneity of variance and normal distribution for
the residuals. Separate GLM models were adopted for each species as this provided the best fit for the
data. We also employed other models (linear regression and logistic regression with a probit scale) and
various transformations (arcsine, square root transformations) but they either did not fit the data, or when
they did, failed homogeneity of variance and normal distribution of error (p > 0.05).

We compared the results for G with those published by Reichman et al. (2006) for salinity.
Therefore, we estimated the osmotic pressure $\psi_o$ (kPa) based on the value range of electrical conductivity
used in Reichman 2006:

$$\psi_o = MRT,$$  \hspace{1cm} (4)

where $M$ is the molarity (mol L$^{-1}$) of the sodium chloride solution, $T$ is the temperature in K (here 298.15
K), and $R$ is the gas constant (J mol$^{-1}$ K$^{-1}$). The corresponding values of $\psi_o$ are presented in Table 1.

2.3 Estimating parameters of the hydrotime model

The hydrotime model was proposed by (Bradford 1990; Gummerson 1986) to “simultaneously
account for both the timing and the extent of germination of a given seed population in relation to its $\psi$
environment” (Bradford 2002). It is defined as:

$$\theta_H = (\psi - \psi_b(P)) t_P,$$  \hspace{1cm} (5a)

where $\theta_H$ is the hydrotime constant (kPa hr), $t_P$ (hr) is the time to germination of percentage $P$ of the seed
population, and $\psi_b(P)$ (kPa) is the base or threshold water potential above which $P$ can still complete
germination. Among the seed population, $\psi_b$ is variable and can be described by a range of frequency
distributions (Mesgaran et al. 2013) of which the normal frequency distribution is the most commonly
used one, defined by its mean $\psi_b(P_{50})$ and standard deviation $\sigma_b$ (Gummerson 1986). Together, these
parameters facilitate to predict the germination time courses, i.e. both rate and extent of germination of the
seed population, at any $\psi$ at a constant temperature (Bradford 2002):

$$t_P = \frac{\theta_H}{(\psi - \psi_b(P_{50}))}.$$  \hspace{1cm} (5b)

In this regard, $\theta_H$ “quantifies the inherent speed of germination, which can vary among species and
physiological states” (Bradford 2002), whereas $\psi_b(P_{50})$ indicates the average stress tolerance of a seed
population, and $\sigma_b$ describes the synchrony in germination timing among seeds in a population. We
estimated these parameters based on the observed values of $t_P$ for each $\psi$, i.e. we plotted the germination
rates ($1/ t_P$ in hr$^{-1}$) as a function of $\psi$ and fitted curves linearly to the observed data (Fig. 4). This resulted
in straight lines with slopes of $\theta_H^{-1}$ and intercepts on the $\psi$-axis corresponding with $\psi_b(P)$. The values of
$\psi_b(P)$ were then fitted to a normal and log-logistic frequency distribution, respectively, and their
distribution parameters were estimated accordingly.
3 Results

Spearman’s correlation coefficient of -0.96 indicated a significant ($p = 10^{-4}$) negative correlation between mean time required for maximum germination and germinability (Fig. 2). Above -750 kPa germinability was remarkably large and mean germination time small. The mean time required for maximum germination ranged between three and five days at water potentials greater than -750 kPa and increased to over seven days at water potentials smaller than -1,000 kPa (Fig. 1b). Likewise, germinability was highest (90% ± 9%) at the water potential corresponding to saturated soil water conditions (0 kPa) and significantly decreased with decreasing water potential ($p = 0.003$) to 65% ± 11% and 20% ± 9% at water potentials of -750 kPa and -1000 kPa, respectively (Fig. 1a). Remarkably, at a water potential as low as -1,500 kPa (permanent wilting point) still 4% ± 1.8% of the seeds germinated. Accordingly, the estimated mean base water potentials were -1,533 kPa and -1,451 kPa for the normal and log-logistic distribution, respectively (Fig. 4), and the estimated hydrot ime constant was 60.7 kPa h (Table 2).

In figure 3 we plotted the germinability of Brigalow seeds in relation to the osmotic pressure based on solutions of sodium chloride as applied by Reichman et al. (2006), and solutions of Polyethylene Glycol as applied in this study. While no significant differences ($p < 0.05$) were observed between the two treatments for osmotic pressures above -400 kPa, germinability was smaller under the sodium chloride treatment for osmotic pressures below -400 kPa.
4 Discussion

The findings of this study allude to the seed water condition of *A. harpophylla*. Together with the initial soil water conditions, these state variables have crucial implications for the rehabilitation of post-mining areas under water-limited conditions.

4.1 Soil and seed water conditions

Initial soil water conditions play a critical role when direct seeding is the primary approach of revegetation on post-mining areas. This is even more important under semi-arid climate, where water is limited due its scarcity or erratic occurrence (Rodriguez-Iturbe & Proporato 2004). Seed germination is triggered by the amount of water the seed can imbibe, which is related to the water potentials of both the soil and the seed (Bradford 2002; Evans & Etherington 1990; Williams & Shaykewich 1971). In this regard, water can only intrude into the seed, if the seed water potential is below the water potential of the surrounding soil material. The empirical results of this study indicate that seeds of *A. harpophylla* are very water stress tolerant, that is, their seed water potential is extraordinarily low. For example, radicles still emerged at a water potential as low as -1,500 kPa (Fig. 1a). Likewise, the mean base water potential of the seed population ranges between -1,533 kPa and -1,451 kPa (Table 2 and Fig. 4). These are remarkable values compared with selected Mediterranean (Köchy & Tielbörger 2007)\(^1\) and agricultural plant species (Watt et al. 2011)\(^2\). Physiologically, the low seed water potential corresponds well with other investigative studies on the water relations of *A. harpophylla* (Doley 2004), which measured foliage water potentials as low as -15,000 kPa (Connor & Tunstall 1968) and shoot water potentials of -7,200 – -6,800 kPa (Connor et al. 1971; Tunstall & Connor 1981; Van den Driessche et al. 1971). Also the seed coat, which is atypically soft compared with other Acacia species, denotes an adaptive mechanism to erratic rainfall patterns of the Brigalow Belt Bioregion to rapidly overcome dormancy if soil water conditions are elevated (Johnson 1964; Reichman et al. 2006; Scott et al. 1984).

The parameter estimates of the hydrotime model (Table 2) play a critical role for predicting the time required to germinate a fraction of the seed population under given soil water conditions (Eq. 5b). In this regard and together with the physiological parameters of the hydrotime model (i.e., hydrotime constant and distribution of the base water potential) the soil water potential governs germination and, consequently, the success of initial vegetation recruitment and early ecosystem establishment. The results of this study show that germinability decreases and the time required for maximum germination increases with decreasing soil water potential (Fig. 1). Moreover, the strong negative correlation between germinability and mean germination time (Fig. 2) underpins how important a rather short germination time is to maximise germination success. Thus, topsoil restoration at post-mining land in the Brigalow Belt Bioregion should target to maximise the initial soil water potential while explicitly considering the erratic character of rainfall patterns in Central Queensland (Audet et al. 2013; Audet et al. 2012). This can be accomplished by optimising soil attributes such as depth, texture, compaction, organic amendments, etc (Arnold 2012; Arnold et al. 2013; Zipper et al. 2013).

Despite plant available water being the primary environmental factor in the Brigalow Belt Bioregion (Arnold et al. 2013), due to hot summers and highly saline mine spoils secondary stressors such as temperature and salinity may also play a critical role for the germination success of *A. harpophylla*, which was tested by Reichman et al. (2006). Their findings indicate no significant trend in germination

\(^1\) \(\psi_b(P_{50}) = -1,390 – -590 \) kPa (based on 36 species)

\(^2\) \(\psi_b(P_{50}) = -1,420 – -1,130 \) kPa (based on 4 species)
among the temperature range of 15 – 38°C. Likewise, seeds of *A. harpophylla* showed remarkable tolerance to salinity up to an electrical conductivity of 30 dS m⁻¹, however, with significant reduction in germination at salinity greater than 20 dS m⁻¹. The authors speculated “it seems unlikely that the reduced germination at 25 and 30 dS/m were due to osmotic effects” (Reichman 2006). While the present study confirms this conclusion for high values of salinity (Table 1) corresponding to osmotic pressure values greater than -400 kPa (Fig. 3), no significant differences could be found between treatments of sodium chloride and PEG (section 2) for small values of osmotic pressure corresponding to high salinity (Fig. 3).

That said, we conclude that under low to moderate levels of salinity the osmotic pressure plays the primary role for germination of *A. harpophylla* seeds rather than the toxic nature of the predominant salt (here sodium chloride), whereas salinity becomes the primary environmental stressor under high salt concentrations. More generally, seeds of *A. harpophylla* seem to germinate quite opportunistically over a broad range of environmental conditions related to temperature, salinity, and water availability.

### 4.2 Alternative rehabilitation approaches

Despite the opportunistic germination capability of *A. harpophylla* seeds, the question remains whether seeding denotes the optimal approach to rehabilitate native Brigalow ecosystems given the hydropedological, climatic, and plant physiological attributes and conditions of the Brigalow Belt Bioregion.

Hydropedological processes are fundamental for the proliferation of Brigalow plant communities (Arnold et al. 2013). The predominant clay soils (Isbell 2002) comprise fine-textured non-cracking Grey and Black Dermosols (Lixisols (Rees et al. 2010; WRB 2006)), and uniform dark cracking Grey and Black Vertosols (Vertisols (Rees et al. 2010)). The latter form “gilgais” (Cowie et al. 2007; Radford et al. 2007; Thornton et al. 2007), which denote ephemeral water storages if filled during intensive storm events. The climate is characterised by erratically distributed rainfall patterns with short and intensive storm events occurring during summer (Cowie et al. 2007), associated with the risk of water logging or soil erosion; whereas the very arid conditions during the winter season generally involve periods of water deficit (Audet et al. 2013; Audet et al. 2012; Dalton 1993). In combination with these erratic climatic conditions the soil properties facilitate rather low soil water potentials on the long-term (Tunstall & Connor 1981), and as a consequence, these landscapes can be colonised by a few plant species only, which is reflected in relatively low species richness and total vegetative biomass (Isbell 2002; Johnson 1980). As elaborated in the previous section, among the species in the Brigalow Belt Bioregion, *A. harpophylla* developed very well adapted mechanisms to proliferate under these harsh conditions. The plants flower only sporadically (Benson et al. 2006) and thus seeds and seedlings are only produced in large numbers during years of extraordinary rainfall (Butler 2007; Johnson 1997). However, *A. harpophylla* is able to reproduce asexually, i.e. through root suckering or sprouting even if aboveground parts of the plant are damaged dramatically, as long as belowground biomass and hydropedology stay intact (Arnold et al. 2013 and references therein). The extent of this vegetative reproduction is most pronounced if trees are young and severely damaged, and under dry conditions (Johnson 1964). In Colorado (USA) circumstances are similar with regard to the re-establishment of vegetatively regenerating Aspen (*Populus tremuloides*) on surface-mined land (Musselman et al. 2012), which initially failed due to severe damage to the root system and thus the limited access to water and nutrients (Shepperd & Mata 2005). However, rehabilitation was more successful when more comprehensive soil-root compartments were transplanted.
from local sources in combination with weed control and light irrigation with non-saline water (Musselman et al. 2012). In this regard, it seems to be crucial to keep the delicate balance between soil attributes (depth, compaction, texture) and root extension in balance. This innovative rehabilitation approach of transplanting the whole soil-root compartment may also be of interest for restoration ecologists engaged with re-establishment of Brigalow ecosystems in Central Queensland to achieve safe, stable, and non-polluting ecosystems (Australian Government 2006).
Acknowledgments

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Collingwood, Australia.


Salinity Laboratory Staff. 1954. *Diagnosis and improvement of saline and alkali soils*. United States Department of Agriculture, Handbook 60.


Table 1

Osmotic pressure $\psi_o$ and NaCl concentration based on the electrical conductivity used in Reichman et al. (2006).
<table>
<thead>
<tr>
<th>Electrical conductivity (dS m(^{-1}))(^a)</th>
<th>NaCl concentration (g kg(^{-1}) H(_2)O)(^b)</th>
<th>Osmotic pressure $\psi_o$ (kPa)</th>
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<tbody>
<tr>
<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>5</td>
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<td>25</td>
<td>11.43</td>
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</tr>
<tr>
<td>30</td>
<td>13.7</td>
<td>-586</td>
</tr>
</tbody>
</table>

\(^a\) – Reichman et al. (2006)
\(^b\) – Salinity Laboratory Staff (1954)
Table 2

Table 2

Parameters estimates of the the hydrotine constant (Bradford 2002), and the log-logistic distribution (scale $\alpha$, shape $\beta$) and normal distribution (mean $\mu$, standard deviation $\sigma$) based on the germination trials of *A. harpophylla*.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimated value</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\theta_h$ (kPa h)</td>
<td>60.7</td>
<td>11.7</td>
</tr>
</tbody>
</table>

*Normal distribution*

| $\mu$ (kPa)     | -1533           | 798            |
| $\sigma$ (kPa)  | 642             | 257            |

*Log-logistic distribution*

| $\alpha$ (kPa) | -1451           | 1.2            |
| $\beta$ (-)    | 1.28            | 1.09           |
Figure 1

(a) Germinability $G$ and (b) time required for maximum germination of *A. harpophylla* in relation to the water potential $\psi$. Error bars indicate the standard deviation across 3 replicates of 50 seeds.
Negative correlation between mean germination time and germinability. Spearman’s correlation coefficient $r_s$ is significantly different from zero ($p = 10^{-4}$).
Germinability $G$ of *A. harpophylla* in relation to the osmotic pressure $\psi_o$ based on solutions of sodium chloride (squares (Reichman et al. 2006) ) and PEG (crosses). Error bars indicate the standard error across 4 replicates of 20 seeds (Reichman et al. 2006) and 3 replicates of 50 seeds for salinity and soil water potential, respectively.
Germination rate in relation to water potential $\psi$ (adapted according to Bradford (2002)). While the slopes of the curves correspond to the reciprocal of the hydrot ime constant $\theta_H^{-1}$, the interceptions with the $\psi$-axis correspond to the base water potential $\psi_b$ for a given percentage $P$ of the seed population. The values of $\psi_b$ can be described by a range of frequency distributions (Mesgaran et al. 2013) among the seeds of a lot (inset). Parameter estimates of the log-logistic ($\alpha, \beta$) and normal distribution ($\mu, \sigma$), and $\theta_H$ are presented in Table 2.