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A pilot study to define and identify future priorities into *Allocasuarina robusta* recovery as part of a community program

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Abstract. *Pearson MW. 2021. A pilot study to define and identify future priorities into* Allocasuarina robusta *recovery as part of a community program. Asian J For 5: 60-70.* The *Allocasuarina robusta* pilot study investigated the process involved to facilitate seed recruitment as part of a threatened species project. Several experiments occurred, each examining a specific attribute in the seed recruitment process. *A. robusta* is a threatened species of national and local significance. The research design would help land managers and communities to conserve *A. robusta.* The investigation aimed to improve seed recruitment in *A. robusta* occurring under natural conditions. The results highlighted several experimental design flaws and identified opportunities to increase community participation as part of the recovery program.

Keywords: Experimentation, germination, Hindmarsh Tiers, Mount Lofty Ranges, pilot study, seed recruitment

INTRODUCTION

Community involvement is a fundamental aspect for aiding environmental restoration; communities contribute by providing labour and time to assist with natural regeneration or ecological reconstruction activities. McGregor and McGregor (2020) described the value and the need for environmental restoration and the intrinsic value to the overall community. Communities can drive a project, but communities that manage areas containing a threatened or rare species cannot perform their role without technical information (Gollan et al. 2012; Roger et al. 2020). These communities can direct research through citizen science programs or other community activities by providing detailed local knowledge (Gollan et al. 2012; Roger et al. 2020).

The purpose of revegetation goes beyond the need to augment natural associations with tube stock or direct seeding to protect biodiversity (Bischoff et al. 2008; Breed et al. 2012; Navarro-Cano et al. 2019). Revegetation is to sustain a population by increasing genetic diversity or stimulating the natural recruitment process without the threat from introduced species (Breed et al. 2012; Padilla & Pugnaire 2006). Revegetation or reconstruction techniques have proven useful for protecting natural communities against further degradation (Breed et al. 2012). While regeneration/ reconstruction measures will aid and facilitate environmental protection through a big-picture perspective (Hobbs 2017). Current regeneration/ reconstruction could all be undone by not understanding what occurs at an individual species level (Breed et al. 2012).

Identifying what environmental cues to measure for seed recruitment in *Allocasuarina robusta* (Macklin) L.A.S. Johnson [Casuarinaceae] means understanding and testing the hypothesis and significance factors (Newman 2008). Newman (2008) described that hypothesis testing and significance testing have a relationship to each other. The relationship can provide misinformation or incorrect inferences when these are not analysed (Newman 2008). A cornerstone of science is the generation of questions; this may involve creating or reaffirming knowledge already known or applying an experimental design differently, none of which will lead to misinformation. Misinformation from experimental design occurs at the execution stage, affecting the results and data analysis (Newman 2008; Symes et al. 2015).

The reporting of experimental design errors or the generalisation of results should not occur in the opinion of Pennock (2004). Undertaking trials is vital for developing appropriate scientific inquiry skills, reducing the possibility of reporting misinformation originating from incorrect experimental design (Symes et al. 2015). A new experimental method or application creates a tendency to focus on procedural components of the experimental design rather than the unexpected outcomes (Chen 2010; Symes et al. 2015). Designing a pilot study should still contain rigour that can test the original research question. As Chen (2010) explains, the concept of an experimental design identifies and engages with the theoretical aspect of testing.

The theoretical aspects of experimental design and its meaning should remain in context (Newman 2008). Maintaining the context and reporting of the results could occur by reporting the experimental design limits (Chen 2010; Newman 2008). Newman (2008) concluded that even if negative results occur, these still require reporting to avoid incorrect inferences from single and isolated tests. A fundamental aspect of understanding restoration ecology is knowing what observations are required and why (Pennock 2004). The current investigation occurs in a simulated environment instead of a field study where Pennock (2004) used second criteria concerning the degree of control exerted in the experimental design. Reporting the experimental design needs contextualisation towards the outcome's size (Newman 2008; Pennock 2004). A pilot study experimental design involves a degree of scalability where procedures and the testing rigour can be measured (Pennock 2004). For example, the results from Navarro-Cano et al. (2019) field plot investigation involved changing the scale to maintain genetic diversity in a species. Pennock (2004) extended the concept of scale to measure the time taken for recording the manipulative experimental data and the experiment's duration.

This pilot investigation aims to demonstrate the importance of experimental design in the restoration ecology. This investigation uses the species *A. robusta,* a threatened species (Minister for the Environment and Heritage, 2006), to test what environmental cues would simulate the populations to regenerate following the parent plant's death.

MATERIALS AND METHODS

Studied species

The climatic conditions favouring *A. robusta* are in the Fleurieu Peninsula's (South Australia) wettest parts (Department for Environment and Heritage, 2007). The Fleurieu Peninsula has a temperate climate with moderately wet winters and hot, dry summers (Armstrong et al. 2003). Rainfall in the Fleurieu Peninsula can range from 400 to 1000 mm depending on altitude and aspect (Armstrong et al. 2003). *A. robusta* grows on soils described by the Department for Environment and Heritage (2007) as infertile acidic soils associated with peat bogs. The soil types range from mottled yellow ironstone soils, gravelly duplex soils and sandy glacial outwash soils (Department for Environment and Heritage, 2007). Hindmarsh Tiers, where *A. robusta* are mainly distributed, has sandy glacial outwash soils (Bickford et al. 2008).

A. robusta occurs in the Kanmantoo bioregion, including the southern Mount Lofty Ranges, Fleurieu Peninsula and Kangaroo Island (Department for Environment and Heritage, 2007). The vegetation is predominantly *Eucalyptus* open forests and woodlands. The habitat for *A. robusta* is the peripheries of wetlands where the mesophytes and hydrophytes meet. The critically endangered Fleurieu Peninsula wetlands communities have legislative protection from the Commonwealth and South Australian State governments (Department for Environment and Heritage, 2007).

A. robusta, a threatened species of the Mount Lofty Ranges, is described as a monoecious shrub with smooth bark (Jessop & Toelken 1986; Wilson & Johnson 1989). The branchlets and the scale leaves of *A. robusta* are glabrous, with the immature scale leaves overlapping (Wilson & Johnson 1989). The female inflorescences produce aggregate fruit from a 3mm long peduncle; these may be sparsely pubescent or sessile on the peduncle (Jessop & Toelken 1986). *A. robusta* seed description is a samara with seed ranging from 5.5 to 6.0 mm in size (Wilson & Johnson 1989).

Pollination in Casuarinaceae occurs by the wind; the bracteoles develop into a fruit that contains a single winged samara seed (Swamy 1948). The female inflorescence develops a woody cylindrical infructescence consisting of whorls of tightly appressed hairs of enlarged floral bracteoles (Pannell & Myerscough 1993). Growth of the floral bracteoles becomes part of the formation of aggregate fruit in *Allocasuarina*. The aggregate fruit is initially hairy and then develops two woody valves with the seed filling the cavity (Swamy 1948). *A. robusta* stores the seed above ground and then releases seed through an environmental trigger (Jessop & Toelken 1986; Quarmby 2011).

Seed collection

A. robusta seeds were collected during September 2017 to February 2018 from 11 sites in the southern Mount Lofty Ranges in South Australia from Hindmarsh Tiers (Figure 1). The collection technique involved collecting aggregate fruit by hand from branches near the base close to the main stem and branches that had not hardened but were still flexible. These sites had additional tube stock planted from other populations within the *A. robusta* range. Sites were selected along an east to the west gradient, and two outlying populations on the north and south. The seeds were collected twice; first a mixture from all 11 sites to form a composite seed collection to test the different simulation techniques and secondly seeds were collected to investigate the populations at an individual level.

Seed examination

Different age *A. robusta* seed were collected and examined. Aggregate fruit from different positions on the *A. robusta* represents a cross-section of age to avoid environmental variability. In each population, sampling occurred on 10% of the population. The 10% sampling relates to the conditions stipulated in the permit from the Department of Environment and Water in South Australia. Each population had a variable number of *A. robusta* from 10 to 112 individuals.

Examination of seed from various populations was done under a dissecting microscope. The initial visual assessment enabled the development of a seed characteristic list. Several characteristics initially included were removed due to being reflective of seasonal variation in the seed. Seasonal variations, as defined by Cochrane et al. (2015), is a cause that affects the seed size (width and length) and the seed's plumpness.

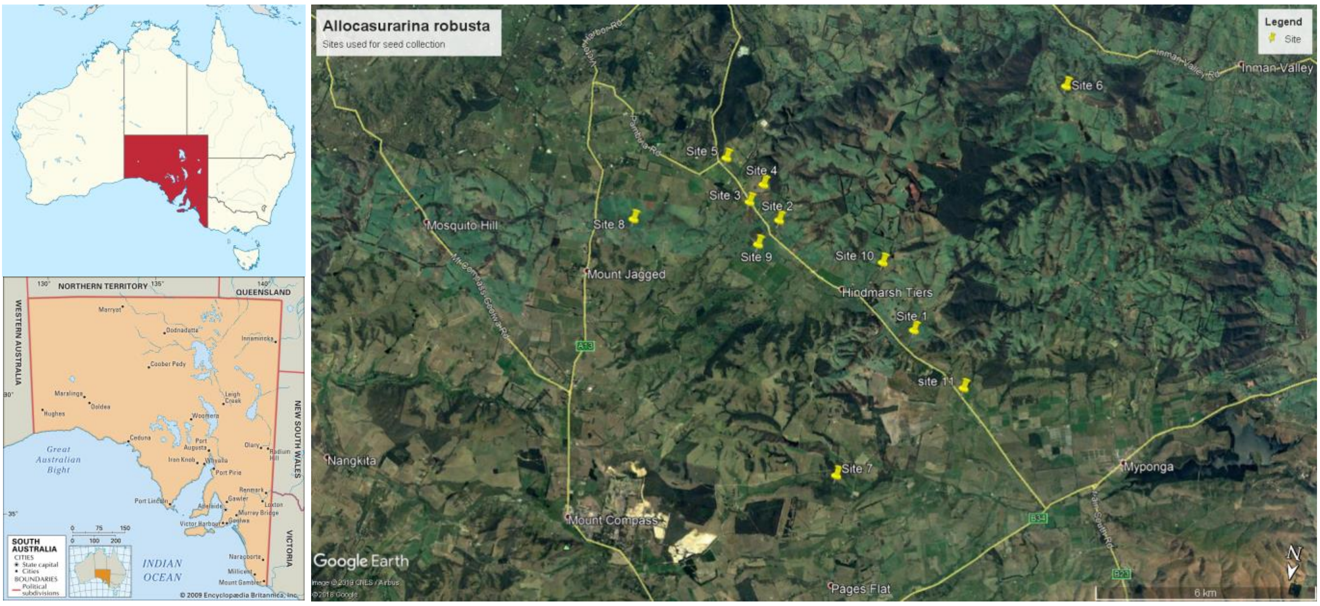

Figure 1. Seed collection sites of *Allocasuarina robusta*

The names assigned to the *A. robusta* metapopulations were the road names or nearby local features, i.e., Hindmarsh Tiers Road had two metapopulations with one sample allocated the road name Hindmarsh Tiers and the second sample named after a nearby local feature Hindmarsh Falls.

The *A. robusta* characteristics list used are as follows:

#1. The geometry of the seed and samara is/
1. seed and the samara are symmetric/
2. seed and the samara are asymmetric/

#2. Midrib in the samara/
1. shows no signs of tapering/
2. shows tapering away from the seed/

#3. Seed surface characterised by being/
1. entire with surface pitting/
2. entire without surface pitting/

#4. Samara colour is/
1. brown/
2. clear/

#5. Seed colour is/
1. brown/
2. black/

#6. The colour pattern between the samara and seed is/
1. ragged without any fading towards the end of the samara/
2. entire and fades towards the end of the samara/

#7. The surface texture of the seed is/
1. rough/
2. smooth/

#8. The placenta connection between seed and fruit/
1. is retuse with smooth edges/
2. is entire with smooth edges/

#9. The shape of the seed is/
1. generally square/
2. generally oval to round/

#10. The shape of the end of the seed is/
1. obtuse to acute in shape/
2. rounded to ovate is shape/

#11. The seed has a colour marking which gives it an appearance of/
1. a single colour without striations (stripes)/
2. striations (stripes)/

#12. Number of teeth/
1. 9 or more/
2. 8 or less/

#13. Number of protuberances on the Aggregate fruit are/
1. single on the back of the bracteole/
2. several on the back of the bracteole/

#14. The shape of branchlets are/
1. rounded or subangular/
2. angular/

#15. The phyllichinia has/
1. a shape that is rounded or subangular/
2. a central groove/

#16. Aggregate fruit are on/
1. pedicels less than 2 mm long or sessile/
2. peduncles 3 – 12 mm long/

#17. Length of teeth/
1. 0.6 – 1.5 mm long/
2. 0.3 – 0.5 mm long/

#18. Teeth bases are/
1. not overlapping/
2. overlapping/

#19. Bracteoles of fruiting cone/
1. thick and convex, often with separate angular or divided protuberances/
2. relatively thin and without any dorsal protuberances/

Competition/nursery

To determine if the presence of either the Burr medic (*Medicago polymorpha* L. [Fabaceae]) and Cocksfoot (*Dactylis glomerata* L. [Poaceae]) contributed towards acting as a surrogate nursery to allow for the establishment of *A. robusta*, a total of 30 punnets sown with the introduced species, i.e., 30 punnets of *D. glomerata* and 30 punnets of the *M. polymorpha*. Fifteen were randomly selected and sown with *A. robusta*, which gave a combination of 15 *A. robusta* and *M. polymorpha* punnets and 15 *M. polymorpha* only punnets. In the repeated method *M. polymorpha* was replaced with *D. glomerata*. The experiment design contained fifteen punnets to act as control with *A. robusta* without any form of competition. Punnets remained in growth boxes for 100 days. The sowing of *A. robusta* did not occur until day 20. The 20-day lag time was to allow the *D. glomerata* and *M. polymorpha* to establish. Response variables were the time of germination and survivorship over 100 days.

Surface litter

As per the observations of Quarmby (2011) dying of *A. robusta* resulted in release of seeds from the canopy. The experiment designed was to mimic conditions of natural seed recruitment in the absence of fire. The experiment used a square squat pot to ensure variability of litter depth while maintaining a proper soil depth to allow a seedlings to establish. Square squats (470ml) procured from Garden City Plastics (https://www.gardencityplastics.com/) were filled with 100ml of growing media and seeds were sown at a density of 30 seed per pot to represent a natural seed rain. The first treatment had a layer of seed placed on the surface litter to replicate natural seed rain. The second treatment placed the seed on the interface between the surface litter and the growing media to represent released seeds and covered with leaf litter (10 mm of leaf litter). The third treatment buried the seed into the litter to a depth of 25 mm, to represent a historical seed release event. The leaf litter were used from *Eucalyptus cosmophylla* F.Muell. [Myrtaceae] (Cup Gum), with the leaves collected from nearby roadside reserves. These were then washed, dried, and exposed to UV light to reduce any possible contamination on the leaves. A fourth treatment sowed the seed on the surface of the growing media without any leaf litter. Each pot was given a number and then assigned to a random location within a four-block experimental design. Each treatment replication were carried out eight times, resulting in use of 24 square squats with six square squats per block. Response variables were the time of germination and survivorship over 100 days.

Growing media

Quarmby (2011) and Bickford et al. (2008) described the *A. robusta* populations' location as centred on glacial outwash. Bradford et al. (2008) described the Fleurieu Peninsula swamps originated from a perched water table. The experiment was conducted to determine which soil type would be conducive for *A. robusta* seed recruitment. The experiment used commercial growing media, which varied in the sand and organic matter ratio. Four treatments of varied soil media were used. These were propagation sand (http://www.brunnings.com.au/propagating-sand-5l.html), orchid mix (https://www.searlesgardening.com.au/products/category/OTNTNCPH-speciality-range/LDEB--searles-dendrobium-orchid-mix-30lt), all-purpose growing media (https://www.debco.com.au/products/all-purpose-potting-mixes/debco-premium-potting-mix) and natural soil, which was determined using a mixture of the soils collected over three sites. Sites were Stipiturus Conservation Park (Site 7), Mt Billy Conservation Park (Site 5) and Hindmarsh Falls (A water reserve managed by the Yankalilla District Council, Site 10) (Figure 1). Sterilization treatment of natural soil were conducted to avoid any competition using solarisation and heating at 200^{o}C for 20 minutes. Twenty *A. robusta* seeds were sown into each punnet. The experiment was conducted in a single block design. Response variables were the time of germination and survivorship over 100 days.

Heat intensity

Fresh cones of *A. robusta* were collected, bagged and then exposed to 100^{o}C for five minutes to allow the fruits to release the seeds. When the seeds were released, they were batched into six groups then exposed to 100^{o}C for various durations.

Exposure length was at intervals of 0 minutes, 2 minutes, 4 minutes, 6 minutes, 8 minutes and 10 minutes. The seeds were sown in in sand-based growing media. The heat exposed groups of seed were replicated four times with thirty seeds sown in a 400 ml punnet. All six treatments were allocated a number and then randomly distributed into a growth box. Response variables included germination over the next 100 days and survivorship.

Heat shock/smoke

The experimental design simulates a fire's effects on the seed described by Mackenzie et al. (2016). To simulate the impact of fire, seeds were sown in wet growing media where treatment was applied and then placed in a three-block design with each treatment replicated three times. Treatments of the seed *in situ* of the potting growing media included heat shock, smoke water, a combination of smoke water and heat shock and a control. The process of simulating heat shock to the seeds occurred by applying boiling water to the seed to act as a form of heat shock (Mackenzie et al. 2016). Heat shock does not necessarily mean exposure to fire in the form of flames but the heat generated by the fire (Mackenzie et al. 2016; Pounden et al. 2014). As *A. robusta* seed protected in the fruit from any direct fire impacts (Clarke et al. 2010). The smoke water treatment comprised of a commercial product from Suregro (http://www.suregro.com/product/regen-2000-smoke-master-liquid-5-litre/) and making up a solution of five litres at the concentration of 0.1 ml smoke water : 10 ml water and applied to punnets through overhead watering.

Seed depletion

Rates of seed loss from within the seed bank have traditionally been quantified by using mesh bags, enabling measurements of seed predation or unviable seeds (Van Mourik et al. 2005). Burying aggregate fruit was considered inappropriate for *A. robusta*. Seed storage of *A. robusta* occurs in an above-ground seed bank.

To measure the seed bank depletion for the *A. robusta*, thirty cones were collected from the west to the east gradient at Hindmarch Tiers (Figure 1). The cones were mixed to create a random source of seed. Grouping of fruits were made at site with six cones per paper bag, providing a comprehensive collection of five sites. Cones were dried and stored in temperatures ranging from 16.7ºC to 19.9ºC, with relative humidity ranging from 84% to 88%. The first bag of seed was sown two weeks after collection, with each subsequent bag sown every four weeks after the initial sowing. Sowing were carried out at a rate of 30 seeds per 400 ml plastic punnet with each treatment replicated four times and then placed in one of four blocks in the growth boxes. The growing media used was a commercial sand-based growing media, including composted organic matter (https://www.debco.com.au/products/all-purpose-potting-mixes/debco-premium-potting-mix). These were monitored daily for germination and survival over 100 days.

Population viability

Seed collection were carried out at eleven of the twenty-four populations of *A. robusta*. Seeds were collected from individual *A. robusta* with 10% of a population sampled with ten cones selected from each individual. The cones were air dried without any environmental controls. Each bag was labelled with date and site information. The seeds were sown two weeks after collection. The seeds were sown in 200 ml punnets using sand-based growing media. Each punnet had thirty seeds sown, with each *A. robusta* collection site replicated four times. Watering of punnets were carried out before sowing and on completion of sowing of the blocks used in the growth boxes.

Growing condition of the seed treatments

Seeds were sown in 200 ml commercial nursery punnet using sand-based growing media unless specified elsewhere. The seed germination occurred in a growth box modelled on the progradation bed designed by Sage Horticulture (https://www.sagehort.com.au/propagation-equipment/propagation-beds/PROPAGATIONTRAYHEATMISTENCLOSURE) using PSI lighting with purple globes. The growth boxes used were 149L clear plastic storage boxes with lighting fixed to the lid. Each box was filled to a depth of 20 mm of playground sand with a heating pad (Medium Seahawk Heat Pad) then covered with a further 20 mm of sand.Heat pads were applied whenever frost was forecasted. Daily data collection on sown seeds were recorded and they were watered every second day with approximately 150 ml of water applied via a mist system to each treatment. Other observational data included recording the seedling's survival viz. assigning a survival category, whether only cotyledons only were visible or central stem was visible with developed scale leaves. The seed collected from *A. robusta* along roadside corridors were carried out from random plants. The seed collection contained a limitation of no more than 10% of fruit collected from any individual and no more than 10% from a population. To meet the South Australian Scientific Permit (A26769-1) from the Department of Environment and Water requirements. Watering used for the punnets consisted of using Adelaide mains water without any purification or treatments. No additional fertiliser or plant growth regulators were used to establish the *A. robusta* seeds unless it was part of the experimental design.

Statistical analysis

Descriptive analyses were carried out using XLSTAT (Mélard 2014). The descriptive statistics included looking at the data's central tendency (mean median, mode, standard deviation and variance). Each experiment was conducted with balance block design to enable ANOVA to identify statistical significance; after that further examination of the data was conducted using RStudio (R Development Core Team, 2010) for linear regression and frequency distribution plots. Single variables formed the basis of the investigation as each of the processes impacted germination due to being a pilot study.

The seed examination used a dissecting microscope at ×45 magnification. Data analysis from collected data was conducted in Delta Ver1.02 (Dallwitz 1993; Dallwitz et al. 2013) and PAUP Ver.4.0a (Swofford 2001). PAUP Ver.4.0a analysed data as an initial branch and bound tree based analysis on parsimony's informative characters. Topological constraints and trees that were unrooted were turned off.

RESULTS AND DISCUSSION

Seed examination

Examination of seed collected from *A. robusta* were conducted before use in the manipulative trials, from the criteria provenance and implications for environmental restoration. The character list design was used to identify any observable differences in morphology and establish a seed provenance for *A. robusta*. The parsimony data generated in PAUP Ver.4.0a showed insignificance for consistency index (0.5926) and homoplasy index (0.4783) towards morphology from the sampled populations.

Competition / nursery

A. robusta sown beneath the *D. glomerata* had only single germination, which is comparable to the *A. robusta* seed sown without competition which also had single germination. Whereas the *M. polymorpha* / *A. robusta* mixture had 12 germinated seeds of *A. robusta*. The number of seeds germinated in the growth box was not enough for statistical analysis, but they showed some interesting relationships with neighbouring vegetation. Not all seeds germinated simultaneously, with the first seed germinating on day 14 and the last seed germinating on day 55 (Table 1) in the *M. polymorpha* / *A. robusta* mixture. The results with *D. glomerata* had only single germination which occurred on day 23 and during the 100 days experimental period, that particular seedling died.

In Table 1, *A. robusta* competition with the *M. polymorpha* only occurred in two of the replications, with the remainder having no *A. robusta* germination. Observation on these treatments showed that the punnets with *M. polymorpha* appeared to be wetter compared to those with *D. glomerata*. Another observation between the two introduced species was that the *M. polymorpha* also had a less aggressive root system than the *D. glomerata*. From Table 1, germination happened over 41 days and it decreased over time.

Surface litter

Eleven seeds germinated over 100 days. Germination began on day 36 and by day 88 it had concluded. Seeds buried to a depth of 25mm recorded no germination, yet the seed sown at the interface between the surface litter and the growing media began to germinate on day 65 and concluded by the 88th day. Seeds sown to simulate seed rain for germination began on day 36 and had concluded by day 85. Simulated seed rain without leaf litter had a more significant number of germinations than the seeds covered with leaf litter (F- value 2.631, DF 6, St. Dev. 19.292, P=0.061).

Growing media

A majority of the germination occurred in the propagation sand, with germination beginning on day 19. Longer germination times were taken on the general-purpose growing media, with limited germination occurring on the composted orchid growing media. In the natural soil mixture, only a single seedling appeared on day 42. The propagating sand had a significantly higher germination rate (F-value 4.494, DF 17, St. Dev. 5.916, P=0.009). The general-purpose growing media (10 germinations, F-value 4.494, DF 16, St. Dev. 1.182) and the composted orchid media (3 germinations, F-value 4.494, DF 18, St. Dev. 1.182) produced the same results with same statistical significance (P=0.01). Even though the composted orchid growing media did have statistically comparable results, the number of observed germinations were less.

Heat intensity

Exposure of the seed to 100^{o}C for 2 minutes intervals and finishing at 10 minutes resulted in 36 germinations. From the observation, 4-minute exposure time produced the highest number of germinations compared to other treatments. Germination began on day 25 and concluded by day 80. It was observed that the more significant exposure time to heat resulted in longer germination times. The control treatment began germinating after 19 days. The observational results indicate that 4 minutes exposure at 100^{o}C was optimum for maximising germination.

Heat shock / smoke

Only three of the punnets produced germinations over the 100 days. Seeds treated with smoke water began germinating 21 days after sowing. From the treatment, only 3/4 produced results, one being germination from the control treatment (Table 2).

From Table 2, the two-treatment exposed to smoke water had much higher germination compared to heat alone or no treatment.

Seed depletion

Sowing and storing seeds at an average ambient room temperature of 23 ^{o}C produced only one germination after 30 days. No other germinations occurred for the next 180 days. Seeds were sown every thirty days for up to 120 days, however, the observation continued upto 180th day.

Table 1. Germination of *Allocasuarina robusta* with *Medicago polymorpha*

Day	Germination	The germinated seedling withered at the cotyledon stage
14	4	0
14	3	0
27	0	1
27	2	0
35	2	0
55	1	0

Table 2. Treated *Allocasuarina robusta* seed germination responses

Treatment	Number of germinations
Smoke Water	3
Smoke Water and Heat Shock	3
Heat Shock	0
Control	1

Table 3. Table results from the population viability experiment

Site	No. of germinations
1	2
2	9
3	2
4	0
5	3
6	0
7	0
8	0
9	7
10	7
11	1

Population viability

On examining eleven populations from west to east gradient of the *A. robusta* over 100-day period, only 31 germinations were found to occur. Population 2 produced 29.03% of germinants. Two other population produced seven germinants (22.58%), and other populations produced between 0-3 germinates. From a geographical interpretation of the data, all three sites with a high germination percentage occurred in the same valley system. Those sites which recorded no germination were in smaller and isolated valley systems.

Discussion

From the present investigation, it could be said that the experimental design was flawed. However, the design flaws provide several vital points for directing future research and identifying the parameters requiring alteration (Chen 2010; Evans et al. 2020; Newman 2008). The notion of reporting results that are flawless or consistent with theory or expectation would weaken application to a meta-analysis (Palmer 2000). Chen (2010) discusses how scientific inquiry does not always occur in ideal circumstances but managed through appropriate variable controls. Reporting on which controls to manage or which variables should be analysed has, as Palmer (2000) indicated, resulted in selective reporting. When considered holistically, the scientific inquiry process and the reporting of either negative or positive results is necessary (Chen 2010; Palmer 2000). When a particular outcome does not occur in the investigative process, these areas allow learning to occur from reflecting on the experiment's hypothesis and design (Chen 2010; Evans et al. 2020; Symes et al. 2015). The current experimental design contained contextual elements from Barritt and Facelli (2001), Abihudi et al. (2020) and Huang et al. (2021). The basis for scientific inquiry is the generation of new information and then explaining the results in the context of current theory (Chen 2010; Newman 2008). By piloting several methods can help identify and redefining the research question, which was the case for the current investigation. The aspect of piloting an experimental methodology and then reporting on the outcome is not new, as Evans et al. (2020) used to refine and identify research areas on seed recruitment. The results' perception indicates a general lack of significance from the current investigation, which supports the need to ensure pilot experiments or trials before larger experimentation is conducted.

From the simulated leaf litter effect on *A. robusta*, the results lacked significance (P=0.061). However, from a similar type of investigation performed by Barritt and Facelli (2001), the leaf litter would impact seed germination. Barritt and Facelli (2001) discussed how simulated, or natural forms would not hinder seedings' emergency. The *Casurarina* litter is like that of *A. robusta* which would be loose and provide no physical effect on seedling emergence (Barritt & Facelli 2001). In the litter experiment conducted for the *A. robusta* and in the investigation by Barritt and Facelli (2001), both experiments only measured a single factor of seedling emergence. Barritt and Facelli (2001) indicated that further investigation was required on the impact of the seedling emergence, including light availability, competition and soil community (e.g., fungi). From the experiment conducted, the p-value produced was not less than 0.05 and in the review of Newman (2008), showed that such result would require retesting or undertaking the experiment again. Nerveless in the context of Newman (2008), the results do not provide confidence towards the significance or the effect size. The lack of confidence from the results produced could, as Pennock (2004) discuss, arise from a lack of replication, but the results contain several similarities to the investigation of Barritt and Facelli (2001).

Replication can improve experimental precision, but replication does not always solve experimental design issues (Pennock 2004). The collection of samples to examine seed morphology of *A. robusta* occurred on an east to west transect through the population. Data collection on seed morphology may not be an accurate ecological indicator of the species' health. Additional data could be collected, including other morphological features, including phenological data related to the species. The addition of phenological data would explain how or when *A. robusta* seed development begins (McDonough MacKenzie et al. 2020). Phenological data would not resolve the sampling aspect, but McDonough MacKenzie et al. (2020) discussed how phenological data supports taxonomy and seed provenance questions. The caveat that needs to be applied is the number of sampling points, and the number of aggregate fruits selected which could allow pseudoreplication to occur (Pennock 2004). Phenological data is not solely focused on when a species flowers but can include when a species is actively growing. Phenological data can increase the taxonomic breath in an investigation by providing supportive information for taxonomy (McDonough MacKenzie et al. 2020). Analysing *A. robusta* seed morphology led to insignificance from the parsimony analysis. McDonough MacKenzie et al. (2020) indicated that phenological data can resolve the parsimony analysis's insignificance. The application of phenological data would increase the diversity in sample data. Applying phenological data would increase data diversity, but care needs to be applied equally to ensure a suitable and representative sample size.

Pennock (2004) indicates that when experimental design lacks sample diversity, it can give rise to pseudoreplication. The investigation focused only on one species (*A. robusta*), but the seed morphology experiment included comparing *A. robusta* relatives seed. Chen (2010) explains how this allows researchers to examine and develop an alternative research question. *A. robusta* surface litter results from current study were comparable to investigation of Barritt and Facelli (2001). Barritt and Facelli (2001) used a different species along with a different environmental habitat. However, future investigations should compare *A. robusta* to a common *Allocasuarina* species from the same environment. The similar type of concept was seen in the study of Abihudi et al. (2020). Identifying which common species to use could come from the investigation of Pearson (2020). The experimentation process tested heat shock, smoke and seed age which can provide vital information for managing a threatened or rare species. Considering study of Abihudi et al. (2020) and the data collected in the present investigation, could indicate a species' conservation trajectory in the Fleurieu Swamps. Germination of *A. robusta* was low in the current investigation. However, Dwyer (2017) conducted a study using species of *Acacia* and found survival of seedlings were low as 0.9% this could represent hundreds of thousands of seeding facilitating the species survival on a per hectare basis. In this investigation a single germinant occurred, meaning it would be difficult to come to the same conclusion as Dwyer (2017) on the number of seedlings required to produce a sustainable population.

The study of Abihudi et al. (2020) comparing a threatened species and a common species. Huang et al. (2021) extended the comparative concept to include an introduced species. The current study investigated the competition/nursery effect, which produced single germination with *M. polymorpha*. The assumption was that the competition for resources would only occur through the interaction between a native species and introduced species. Huang et al. (2021) indicated that competition could occur between two native species and the competition/nursery effects take place through the time taken for germination and the speed at which the species establishes. Observations from the current investigation can be related to concepts discussed in Catterall (2019), which highlights the role of nurse plants in restoration ecology and the current results will require further investigation to be more conclusive and definitive. Barritt and Facelli (2001) identified several related factors that impact germination (i.e. the interrelationships between a nurse plant and the species under study), all of which require further investigation. Understanding the role or relationship of a nurse plant with *A. robusta* will require further investigation. Lozano et al. (2020) explored the nurse plant relationship and role in the recruitment process. Lozano et al. (2020) identified that the main contributing factor for establishing a nurse plant relationship is soil, but in *A. robusta*, the evidence collected is inconclusive and requires further investigation. The current investigation had single germination, which poses several questions, did this occur by chance or was it through soil amelioration, as was the case in the study of Lozano et al. (2020). Alternatively, a single germinant's survival reflects long-term recruitment strategy of *A. robusta*, which also occurs in other species (Dwyer 2017; Navarro-Cano et al. 2019). Navarro-Cano et al. (2019) investigated the species' long term recruitment trajectory in a different genus.

The reporting of the investigation results and the comparison with other investigation (i.e. Abihudi et al. (2020) and Navarro-Cano et al. (2019) requires a caveat to avoid misinformation to give it a theoretical basis. Palmer (2000) termed investigation, which used different systems and species quasi replication. Identifying and comparing data against other species or different systems is often used, or comparative analysis from a data subset used as a means of justification (Palmer 2000). Palmer (2000) would not entirely dismiss the role that quasi replication can have in science. Chen (2010) identified that comparing but not analysing the investigation is not a true reflection of the hypothesis. For instance, the design of the heat intensity and heat shock/smoke experiments should demonstrate a significant germination event post fire for seed recruitment in *A. robusta*. The reported results were not conclusive to support or dismiss the hypothesis. A simulated fire used in the experimental design contained no comparative analysis with another species. Cury et al. (2020) compared species under the same condition which provided results with meaning and a theoretical context. In the current investigation, the absence of comparison between *A. robusta* and another species provides an opportunity for further investigation.

Replication needs to conducted under the same conditions, while comparison between common/ threatened or threatened/invasive can be made. The results from the current investigation examined the metapopulations of *A. robusta*. The results were not conclusive; the appearance of pseudoreplication could occur; as Pennock (2004) describes, the hypothesis was to determine the viability of metapopulations for natural regeneration. The method selected was like the method used by Costa e Silva et al. (2019) for examining the provenance of a Tasmanian *Eucalyptus* species. Costa e Silva et al. (2019) compared *Eucalyptus* provenances through replication within a controlled growing environment and a common garden experiment, while experiments on *A. robusta* was conducted only in a controlled growing environment. The current investigation requires a comparison to aid the recovery of *A. robusta* further.

From the data collected, several opportunities exist which can further aid the recovery of *A. robusta*. The community's role in collecting data or identifying new metapopulations of *A. robusta,* particularly on private land identified by Quarmby (2011). A strategy described by Breed et al. (2012) would provide the ideal means to achieve success through a tube stock program to improve seed recruitment practices. The caveat placed on the strategy needs to be evidence-based for the ecological community and each species used (Breed et al. 2012).

The relationship between community-driven action and science can support the recovery of *A. robusta*. From the investigation conducted, further investigation into seed recruitment of *A. robusta* is still required. Breed et al. (2012) discuss genetic diversity and variability as part of the seed collection strategy. Through population genetics, this would aid in understanding whether areas of seed provenance exist or not. The genetic data can aid and inform the community on how to progress the recovery efforts. Informing the professional's recovery efforts is not a one-way process, but a two-way process as the community can inform the professional to direct and support the research (Gollan et al. 2012). Gollan et al. (2012) identified that data collected from volunteers (community) is comparable to professionals' data. Using the findings from study of Gollan et al. (2012) and applying them to the *A. robusta* recovery project could mean that replication for common garden experiments could be made in various field locations where citizen science monitoring are taking place. For instance, the investigation involving a competition/nurse plant or leaf litter could be an ideal project that the community could undertake as a common garden experiment in collaboration with the professionals. The collaborative work undertaken as part of a citizen science project as part of the community engagement can produce valuable data aiding the recovery efforts of a threatened species (Roger et al. 2020).

Defining the role of citizen science is essential for ensuring the success of the program. Community involvement in threatened species management would involve ensuring that some degree of quality control would be necessary over the data collection, which can be made through benchmarking activities for data collection (Gollan et al. 2012). Design consideration for increasing community involvement would, as Gollan et al. (2012) described, need to have simple and easy to use data collection tools. Gollan et al. (2012) described several case studies where presence/absence data collection tools or flipbooks were used to identify species present. Such ideas of Gollan et al. (2012) may be related to the *A. robusta* recovery project, particularly monitoring disturbance and natural regeneration. As can be seen, the experimental design problems allow space for rectification, which are a small investment that can guide the future direction of recovery efforts. Pilot studies can provide a means to refine or better identify the issues associated with species recovery. In the current investigation, the experimental design was refined from identifying problems at the pilot stage but more importantly, it led to the identification of new opportunities to aid *A. robusta* recovery.

In conclusion, interpreting the results provides some general inferences only but can provide an opportunity to reflect on the experimental design for *A. robusta* recruitment. Comparing *A. robusta* seed germination to a species of least concern would facilitate the species recovery process. The concept is no different from the investigation conducted by Abihudi et al. (2020). Abihudi et al. (2020) demonstrated the benefits of small discrete experimentation on a species could improve species management. *A. robusta* inhabits an environment considered to be prone to disturbance. The pilot investigation identified experimental design shortcomings, which translates to requiring further investigation but focussing on the essential environmental cues. For the recovery of *A. robusta* to be successful, community involvement is essential which can be applied from the lessons learned through experimentation and research at the pilot stage.

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