AUSTRALIAN FLORA FOUNDATION

RESEARCH GRANT

PROGRESS REPORT

Improving seedling establishment in Endangered *Whibley wattle*

Figure 1. Whibley wattle in flower (September 2020) with EPLB Archie and Fred (L – R).

Key AFF Special Interests addressed:

1. Conservation of Australian plant diversity, particularly where threats from climate change

3. Rare and endangered plants.

Research Organisation

The University of Adelaide (UoA), in partnership with Eyre Peninsula Landscape Board (EPLB), & Department for Environment and Water (DEW).

RESEARCH UPDATE HIGHLIGHTS

Improving seedling establishment in Endangered *Whibley wattle*

Whibley wattle (*Acacia whibleyana*) is nationally endangered and one of Australia's 30 Priority Plant Species. This large shrub is restricted to the Tumby Bay area on South Australia's Eyre Peninsula. One of its greatest threats is the lack of recruitment due to altered disturbance regimes. Our experimental trials bring together land managers and research scientists in a partnership between Australian Flora Foundation, Eyre Peninsula Landscape Board, and The University of Adelaide. Together we **aim to discover the influence of mechanical soil disturbance, seed supplementation, and water availability on *Whibley wattle* (1) germination, and (2) seedling establishment.**

Twenty five Whibley shrubs were established as plots for three experiments; each plot has four subplots and eight quadrats for Experiments 1 (±soil disturbance with rotary hoe, ±watering to long-term monthly average), 2 (±water, ±disturbance) and 3 (±seed supplementation, ±disturbance and ±water). Total quadrats = 200. Our trial was planned to begin April 2021 after breaking Autumn rains but, due to Covid-19, was delayed until late June 2020. We then monitored all plots monthly until 31 December 2020.

Preliminary results include:

- increasing expertise and confidence of land managers in strategic monitoring
- increasing recording of Whibley morphology, phenology (e.g. seed set), and health (e.g. galls, dieback)
- only 3 seeds germinated naturally pre-disturbance – 2 were surviving in Feb 2021
- almost 50 seeds germinated post-disturbance – but none survived to January 2021
- we continue to monitor the 2020 natural germinations and are watching for further post-treatment germination after 2021 Autumn rains.

1. GENERAL INFORMATION

1.1. Project Title

Soil disturbance trials to improve germination and seedling establishment for the Endangered Whibley wattle (*Acacia whibleyana*)

1.2. Project Duration

This is a two year project (1 February 2020–31 December 2021). Support from Australian Flora Foundation is pivotal.

1.3. Total Grant Approved

$19,794.

1.4. Project Aims

Whibley wattle (*Acacia whibleyana*) is a national priority within the Threatened Species Strategy's 30 Priority Plants, and the only priority Acacia that occurs in southern Australia (Commonwealth of Australia 2019). One of the greatest threats to a self-sustaining population of Whibley wattle is the lack of recruitment due to inappropriate disturbance regimes (UoA 2019). This project therefore **aims to**:

- co-design a disturbance trial with research scientists and land managers
- **test the relative influence of mechanical soil disturbance, seed supplementation, and water availability on *Whibley wattle* (1) germination, and (2) seedling establishment**
- test the logistic feasibility of mechanical soil disturbance as an alternative to ecological burning for promotion of *Whibley wattle* germination and seedling establishment.

2. METHODS

2.1. Research location and study site

The field experiment was conducted in the main Salt Lake stand (34°27'04.2"S 136°02'08.1"E; Figure 2) within one of the most extensive and representative subpopulations, Salt Lake (Figure 2). This stand was selected to meet seven criteria identified during planning (*Section 2.2. Project Full Application*): (1) number of individuals present – large stand with over 50 individuals at start date, 1 February 2020 (2) age structure/demographics – enough reproductively mature individuals; (3) genetic diversity; (4) healthy individuals with minimal dieback; (5) availability of previously collected viable seed (confirmed by SA Seed Centre); (6) accessibility for equipment (rotary hoe for disturbance trial) and volunteers to assist with watering and monitoring; (7) landholder support to ensure protection from habitat clearance; and protection from grazing – already fenced to protect from stock and rabbits (kangaroos not known to be present).

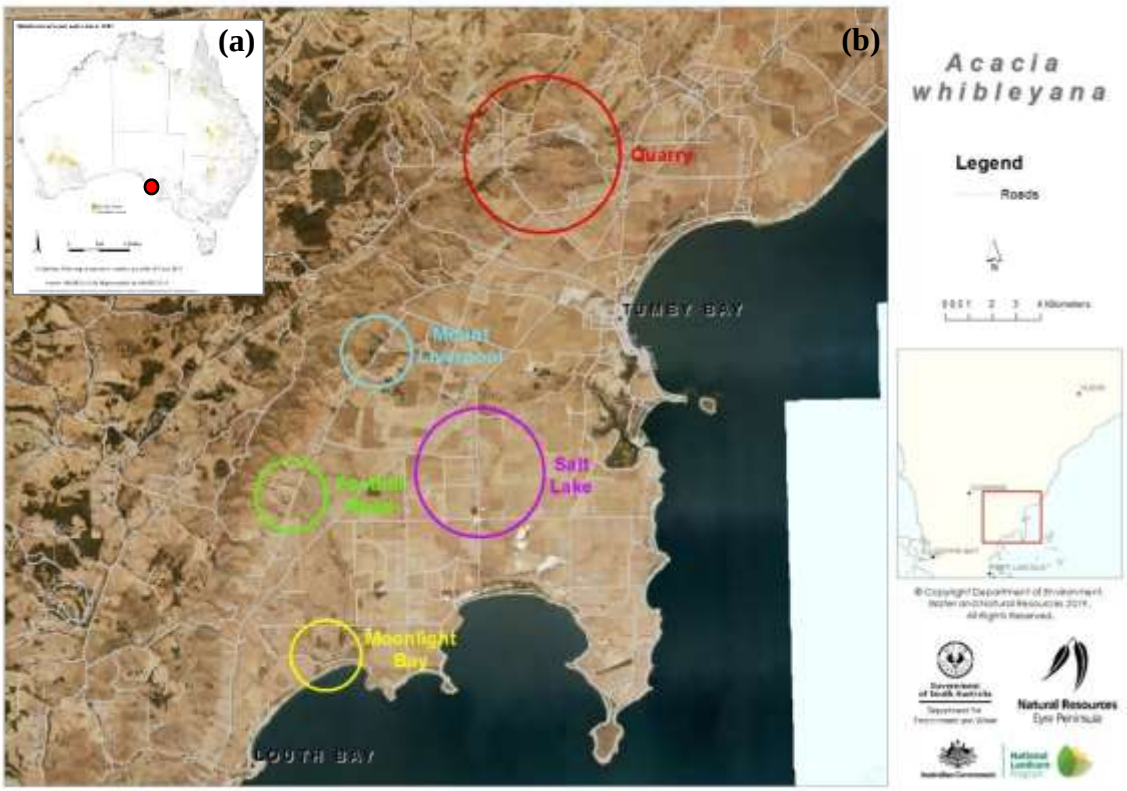


Figure 2: Location of Endangered Whibley wattle (*Acacia whibleyana*) population, and the five disjunct subpopulations indicated by coloured circles (b). Project research area and Salt Lake site indicated in **purple**.

2.2. Experimental design

To assess the influence of soil disturbance on Whibley wattle germination, and influence of watering on establishment, we used a field experiment with split plot design.

- Experiment 1: relative influence of soil **disturbance** and water on **germination** from seedbank
- Experiment 2: relative influence of **water** and disturbance on **establishment** from seedbank

To maximise the likelihood of successful germination from EPLB's in-kind investment, we paired each experiment with seed supplementation.

- Experiment 3: relative influence of **seed supplementation**, water supplementation and disturbance on **germination and establishment.**

Our experimental design also included **preliminary assessment** of the relative influence of other abiotic conditions (elevation, light availability (canopy cover), soil salinity and soil pH) to inform future translocation site selection, and biotic conditions (density of non-native plants, litter cover) to inform management actions. See **Appendix 1** for detailed methods of all three experiments; here we provide a summary of our approach.

2.3. Plot selection

Twenty five experimental plots were selected, centred around healthy, reproductively mature Whibley wattles (one shrub, or dense cluster of shrubs, per plot), and marked with unique identification (numbered brass tags). For Experiments 1 and 2, half of each plot (subplot) was randomly assigned to one of two subplot treatments (Figure 3):

- Disturbed (+ mechanical soil disturbance)

- Control (– disturbance).

Each Disturbed treatment subplot was divided into two 50 x 50 cm quadrats (n = 200), and randomly assigned to one of two quadrat treatments:

- Watered (+ water)
- Unwatered (– water).

Disturbance Trial

The disturbance trial was planned for mid-April 2021, following breaking Autumn rains to optimise *in situ* germination of *A. whibleyana* (Manfred's paper and GT, pers. comm.). However, due state-imposed Covid-19 travel restrictions, the trial had to be delayed until late June 2020.

All 25 plots were monitored monthly until 31 December 2020 for Experiment 1 (germination), and will be monitored at least until December 2021, but ideally until 2023 (subject to further funding), for Experiment 2.

For Experiment 3, we paired all Experiment 1 and 2 with replicate quadrats. For each subplot, Whibley seeds were added to the left quadrat (facing inwards to the shrub centre). The right hand quadrat had no seed addition. All 25 plots will be monitored at least until December 2021, but ideally until 2023 (subject to further funding).

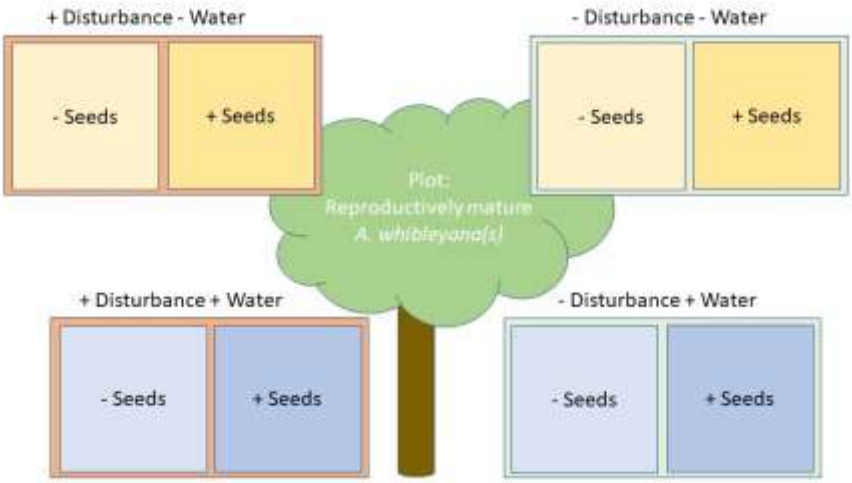


Figure 3: Experimental design of **one plot** with four subplots and eight quadrats for Experiments **1 (±disturbance**, ±water), **2 (±water**, ±disturbance) and **3 (±seeds**, ±disturbance and ±water). Total plots = 25; total quadrats = 200.

2.4. Statistical analyses

We are using a mixed modelling approach to investigate the relative influence of covariates, including any interactions between them, on Whibley wattle germination and seedling establishment. A priori predictions, and mixed models, were developed for the anticipated influential variables.

Table 1: Example of a priori models. Null and predicted models for Experiment 1.

Model number	**Covariate structure**
A priori model 1 (NULL)	Awsdl ~ 1 + random(subplot|whibley.plot)

A priori model 2 (full candidate model):	Awsdl ~ treatment.disturbance + vegstruct.prop + vegstruct.species + pHw0_6 + EC0_6 + random(subplot|whibley.plot)
A priori model 3	Awsdl ~ treatment.disturbance + random(subplot|whibley.plot)
A priori model 4	Awsdl ~ treatment.disturbance + vegstruct.prop + random(subplot|whibley.plot)

Power analyses were undertaken to identify likelihood of detecting germination response at expected rates (based on literature), All data are checked through exploratory data analyses, and analysed with generalised linear mixed models that take into account our nested split plot design. Variables include:

- response variable(s): number of *Whibley wattle* seedlings (density), seedling productivity (no. of phyllodes, juvenile or mature), proportion of native versus non-native seedlings (richness, diversity, invasiveness).
- covariates: elevation, genetic architecture (where possible), light availability (canopy protection), pH, and treatments (disturbance absent/present, water absent/present, seeds absent/present), random variables to account for spatiotemporal replication of repeated monitoring of the same quadrat nested within subplot and plot.

Table 2: Response variables surveyed. Details of explanatory variables are provided in Appendix 1.

Variable	Description	Type
Response variables		
Awsdl	No. Aw seedlings present in quadrat	count
Awsdl.produ	Aw seedling productivity = no. leaflets	count
Awsdl.phenol	Aw seedling phenology = no. leaflets	count
Awsdl.status	Aw seedling status	categorical

Please refer to **Attachment 2** for a GANTT chart summary of our project milestone completions and progress.

3. PRELIMINARY RESULTS

3.1. Co-design disturbance trial with research scientists and land managers

We have co-designed and co-managed the project to ensure our experimental design and methods address shared priorities with EPNRMB. Outcomes include:

- Referred to literature review for ecology of whibley and conservation priorities
- In-field discussion identified priority of trialing soil disturbance (and potential use of rotary hoe) Several discussions during development of application to confirm EPNRMB capacity and priorities
- Discussion in-field during plot selection phase – reiterative process together to review long-term EPNRMB priorities (whibley recruitment and establishment to achieve self-sustaining subpopulations) and capacity (landscape-scale management with minimal hand-held intervention

for individual plants ie. likely to use large machinery to potentially turn soil and seed, and not be able to do any followup tamping of soil or weed followup)
- Increasing expertise and confidence of EPLB staff in strategic, experimental monitoring
- Increasing observation and recording of Whibley wattle morphology, phenology (e.g. seed set, January 2021), and health status (e.g. gall phenology and associated absence/presence of dieback, January 2021).

3.2. Experiment 1: relative influence of soil **disturbance** and water supplementation on **germination** from natural seedbank

Preliminary results show that only one seedling, out of almost 50 that germinated after June 2020, has survived to January 2021.

3.3. Experiment 2: relative influence of **water** supplementation and disturbance on **establishment** from natural seedbank

Unfortunately, as only 1 of 50 germinants survived, we are unable to monitor seedling **establishment** following disturbance and/or water supplementation. However, it is quite conceivable that soil disturbance could trigger more germination, with potential for establishment, in subsequent years.

In contrast, 2 out of 3 seedlings that had germinated naturally prior to June 2020 (autumn 2020), are still alive, actively growing, and appear to be healthy. Our monthly monitoring is tracking the progress of the remaining pre-disturbance seedlings towards potential establishment.

The poor survival rate observed in our disturbance trial is most likely attributable to the forced delay of the soil disturbance from mid-April to late June. The trial was planned for April, based on our literature review, when soil temperatures were higher and to occur within 1 week after >20 mm season-breaking rains. We suspect that seedlings germinating earlier in the year would have grown stronger and had a much greater survival rate through spring and summer.

For this reason, we are seeking additional funding to extend the monthly monitoring throughout 2021 and 2022. This would enable us to detect any new germinants that emerge in autumn and winter of 2021 and monitor germinant survival and establishment through to 2023.

3.4. Experiment 3: relative influence of **seed supplementation**, water supplementation and disturbance on **germination and establishment**

No preliminary results available as minimal germination occurred, regardless of with or without seed supplementation, but no germinants survived. Mixed modelling with zero inflated data will be required to assess the influence of seed supplementation.

3.5. Logistic feasibility of mechanical soil disturbance as an alternative to ecological burning

Experiment 1 confirmed that using a rotary hoe for soil disturbance is a logistically feasible alternative to ecological burning. The rotary hoe hire was affordable and the machine able to be maneuvered successfully around the experimental plots.

The germination response, however, was not substantially higher in the disturbed versus undisturbed plots. Further research is needed to identify what is limiting germinant survival before further soil disturbance trials are considered.

3.6. Additional outcomes

In-kind contributions continue to be provided by EPLB, SA Seed Centre and UoA.

- EPNRM:
  - Project planning and arranging equipment, permits, accommodation, vehicle, ongoing project development and planning to adjust to low survival, etc. (1 staff)
  - Seed collection in 2019 (1 staff)
  - Materials – wooden stakes, flagging, GPS, etc. ($200)
  - Transport (use of EPNRM vehicle in January) ($250)
  - GT's time on site with us (Dec 2019, Jan 2020 (2 days), June 2020 (3 days) (1 staff)
  - Car Hire (5 days in June) ($500)
  - Gate installation and fence repair (cost? + staff hours)
  - Interpretive gate sign to help protect experimental Whibley shrubs and plots (and promote Whibley conservation)
  - Soil collection (3 staff – 1 half day x3)
  - Soil analyses ($5K)
  - Monthly watering – rainfall dependent; use of water truck and staff time (3 staff)
  - Ongoing monthly monitoring at site (1 staff)

- SA Seed Centre:
  - Seed viability testing

- University of Adelaide:
  - Project planning, admin (OHS forms & permissions, booking flights, accommodation, purchasing equipment etc.)
  - Grant administration (contracts, Covid updates etc.)
  - January flights and accommodation
  - December field trip, unpaid
  - January Field trip, unpaid – 2 days, 2 staff
  - Methods write-up, data entry and field trip planning (Jan trip)
  - Steve Delean – statistics advice – 1 hour
  - Research on seed germination & viability in other Acacias
  - Statistical modelling to optimize seed numbers
  - June field trip unpaid hours: 2 days + overtime, 2 staff
  - Media releases and interviews (ABC Radio, ABC Radio blog).

3.7. Emerging application of this work to benefit other Australian native plants

Acacia whibleyana is found within two EPBC-listed communities (*Eucalyptus odorata* grassy woodland (CE) and *Eucalyptus petiolaris* woodland (E)), and in habitats that support other nationally or regionally threatened species. In the long-term, restoring the natural recruitment of *Acacia whibleyana* in these communities has the potential for ecosystem-level benefits such as improved pollination and habitat resources.

The findings from our trials are relevant for other threatened species, including other Acacias. At the national level alone, 75 *Acacia* species are listed as critically endangered, endangered or vulnerable. For many of these, uncertainty around their response to disturbance is recognised as a key threat. The support of this AFF project has encouraged us to develop a manuscript for the International Biological Flora series, Journal of Ecology, on Acacia whibleyana as a model species for threatened Acacia internationally. Professor Anthony Davy, Series Editor, has accepted our proposal and we are finalizing the draft for preliminary submission. Our trial and manuscript findings may also benefit other obligate seeders where human-mediated alterations to natural disturbance regimes (particularly avoidance of disturbance) is limiting recruitment and may risk depletion of the soil seed bank.

4. MILESTONES

Amount of funds awarded:

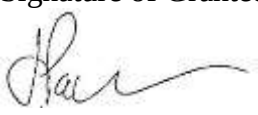
Total funds approved **$19,794** (this amount includes GST) payable as follows:

On signing of contract:	$9,962	COMPLETED
On provision of suitable progress report	$7,853	available 1/1/2021: PENDING
On receipt of a suitable final report:	$1,979	available from 1/1/2022: SEE BELOW
Total	$19,794	

Further to our email correspondence with Peter Goodwin in December 2020, we request an extension of time to submit our Final Report by 31 October 2023. This additional time would enable us to continue monthly monitoring of the 25 Whibley plots (2 surviving pre-disturbance seedlings and any new natural germinations) until 2023.We plan to apply for supplementary funding for this further work from the current 2022 grant round.

Progress Report prepared by Jasmin Packer and Renate Faast (UoA), and Geraldine Turner (EPLB).

Signature of Grantee



Date 31/01/2021

Appendix 1

Acacia whibleyana Disturbance Trial Methods (DRAFT)

Project team: Renate Faast (UoA), Jasmin Packer (UoA), and Geraldine Turner (EPNRMB)

Site selection

The Salt Lake Main stand within Salt Lake subpopulation was selected as the survey site based on the following criteria:

- existing fencing from grazing pressure (kangaroos, rabbits and stock; minor repairs to the fence were provided as in-kind by EPLB prior to the trials commencing)
- large stand (>20 individuals) within a large subpopulation (>2 stands) to ensure trial wouldn't compromise viability of the subpopulation
- existing evidence base of past monitoring and genetic data to inform experimental design
- accessible for disturbance treatment (rotary hoe for trials, tractor and slasher for future management if disturbance trial recommends landscape-scale disturbance)
- accessible with public access for volunteers to carry out ongoing post-disturbance watering and monitoring.

Plot selection

Healthy, reproductively mature Whibley shrubs were selected based on four criteria:

- large enough to accommodate 4 subplots (minimum of 5.5m canopy circumference) around the drip line
- minimum of 5.5m available that avoided removal or damage to a large proportion of understory shrubs or ground covers, and also avoided interfering with adjacent experimental plots.
- less than 50% canopy dieback
- Note: Whibley shrubs growing together in a dense cluster (i.e. formed a continuous large canopy) were included as single plot.

A total of 32 suitable plots were identified. Of these, 25 plots were randomly selected (www.random.org random sequence generator) for the Disturbance Trials.
Note: one of these adults was dead by June 2020, and the plot was replaced with a randomly chosen plot identified earlier (out of the original 32).

Each plot was marked with a unique identifying brass tag (numbered 1801-1825), attached with wire to a branch of the shrub. These tags are fire-proof.

Canopy characteristics were recorded for each of the 25 *A. whibleyana* plots were surveyed (ie. measurements for clusters were across the entire plot): maximum height, minimum width, maximum width, dieback.

Four subplots in each plot

- Each subplot consists of two 50 × 50 cm quadrats adjacent to each other. This design was decided upon (as opposed to 8 individual subplots each spaced 50cm apart) because there were too few plots available that were large enough (minimum 8 m canopy circumference).
- The center of each subplot was positioned to align with the canopy dripline, such that half of the subplot was beneath the canopy and half was outside. This allowsus to assess the importance of canopy cover for seedling recruitment, and also minimized disturbance of Whibley roots beneath the shrub.
- Randomization: The position of the first subplot was selected using a randomized compass bearing (www.random.org random sequence generator). Subsequent subplots were positioned

at intervals of 150 cm (in a clockwise direction), unless this point interfered with existing understory plants or adjacent plots in which case the subplot was moved further along.

- Each subplot was marked with Galvanised nails (Figure x), and a labelled metal tag nailed into the ground, indicating the Plot Number, Subplot No. and Treatment.

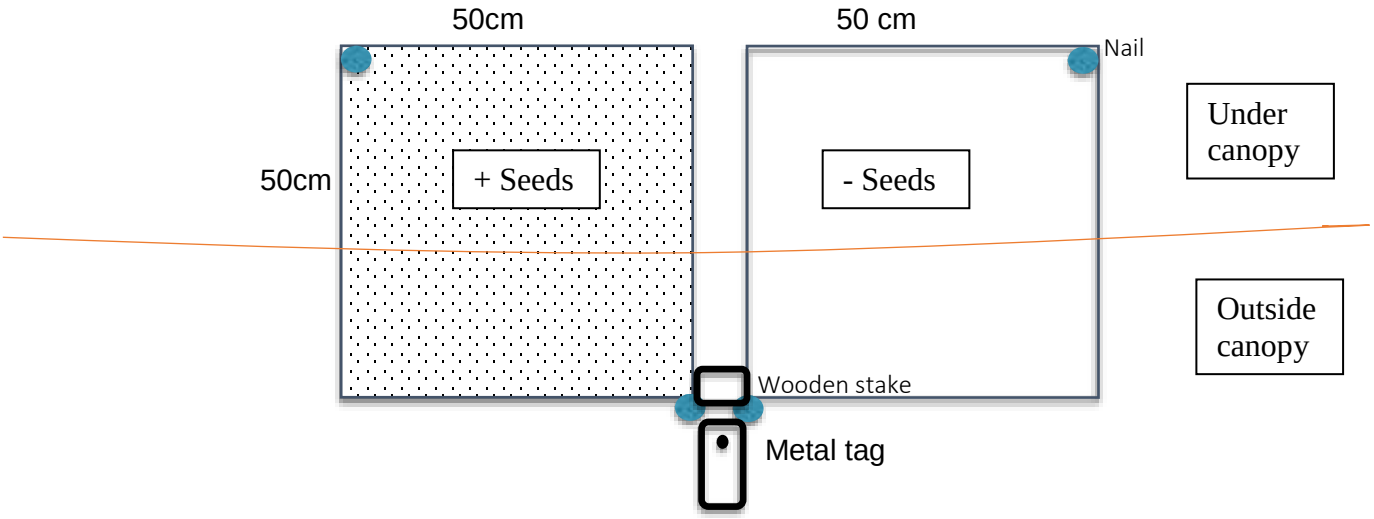


Figure 1 Example of subplot design and setup, for Experiment 3: +seed (L) and –seed (R) quadrats. Aqua circle indicates nail.

Assigning experimental treatments

- Each subplot was randomly assigned to one of 4 treatments:

Label	Disturbance	Water
+D +W	Yes	Yes
+D -W	Yes	No
-D +W	No	Yes
-D -W	No	No

- Each subplot was labelled with a metal tag nailed into the ground, indicating the Plot Number, Subplot No. and Treatment (e.g. 1801.1 +D+W)
- After the disturbance was conducted, additional nails were inserted at the oblique corner to enable accurate relocation of the quadrat frame.
- Labelled wooden stakes were also added to clearly mark each subplot (in between the 2 quadrats), and watered subplots marked with spraypaint (to assist watering treatment).

Pre-disturbance assessment of Ground cover and Canopy (22 – 23rd June 2020)

- One 50 x 50cm quadrat (stainless still grid) divided into 25 cells (5 rows of 5) was placed on the ground
- Within each quadrat, we counted the number of cells containing:
  - *A. whibleyana* germinants
  - Litter (<5% litter within cell) – does not include dead but attached vegetation
  - Moss
  - Bare ground

- Exotic species
- Native species
- Projected canopy cover – looking down over centre of quadrat

- Recorded the native and exotic species present in each quadrat, and the canopy species.

Table X: Explanatory variables surveyed - abiotic

Variable	Description	Type
bearing	Bearing of quadrat in relation to plot centre	ordinal
plot.easting	GPS coordinates of Plot (WGS84 UTM, Zone 53H)	numeric
plot.northing	GPS coordinates of Plot (WGS84 UTM, Zone 53H)	numeric
pHw0_6	Soil pH, 0-6 cm deep, 1:5 Water	numeric
pHw10_20	Soil pH, 10-20 cm deep, 1:5 Water	numeric
pHCa0_6	Soil pH, 0-6 cm deep, 1:5 CaCl2	numeric
pHCa10_20	Soil pH, 10-20 cm deep, 1:5 CaCl2	numeric
EC0_6	Soil EC 0-6 cm deep	numeric
EC10_20	Soil EC 10-20 cm deep	numeric

Table x: Explanatory variables surveyed- biotic

Variable	Description	Type
plot.height	Max height (cm) of Adult A. whibleyana	numeric
plot.maxwidth	Maximum width (cm) of plot around adult A. whibleyana(s)	numeric
plot.minwidth	Minimum width (cm) of plot around adult A. whibleyana(s)	numeric
plot.noindiv	Number of A. whibleyana individuals in plot (adult & juveniles forming dense cluster)	count
Fvegstructure	Plant life forms; L = Litter; M = Moss, E = Exotic, B = Bare ground; N = Native; C = canopy projected	categorical
vegstruct.prop	Proportion of cells with plant cover (out of 25)	beta reg?
vegstruct.species		
Native species	Native species present. Poa = Poaceae (e.g. Austrostipa spp.); Dc = Dysphema crassifolium; Et = Enchylaena tomentosa; Nb = Nitraria billardierei; Ss = Senecio species seedlings (e.g. S. glossanthus); Aw = Acacia whibleyana seedlings; Ti = Tetragonia implexicoma; S/T = Suaeda australis or Threlkeldia diffusa; Sam = Samphire species; En = Einada nutans; Pa = Pittosporum angustifolium; O = Other (Atriplex semibaccata, Rhagodia, Crassula spp., Nitraria billardieria, unidentified species)	categorical
Exotic species	Ms = Moraea setifolia; AG = annual grasses (e.g. Avena barbata); BL = broad leaved (e.g. Sonchus spp., Hypochaeris spp., Crassula sp.; Anagallis sp.); Aa = Asparagus asparagoides (Bridal creeper); Cheno = chenopod seedling; Es = Erodium sp. (possibly E. botrys); Mc = Mesembryanthemum crystallinum; Af = Asphodelus fistulosus; BH = Branched herb?; Mn = Mesembryanthemum nodiflorum;	categorical

Canopy species	Aw = Acacia whibleyana; DWD = dead woody debris; Ma = Melaleuca acuminata; Pa = Pittosporum angustifolium	categorical
Awsdl.canopy0_20	Canopy Pole touches above Aw seedling (0-20 cm; 20-40 cm etc)	count
native.germ	Number of other native germinants	count
native.numbsp	Number of species of native germinants	count
native.sp	Species of native germinants	count

Pre-disturbance Coarse woody debris removal

- Coarse woody debris (>10mm) was removed from all quadrats if deemed necessary for the disturbance treatment (rotary hoe operation).
- In Disturbed quadrats, remaining litter was left in place, and "mulched" into the disturbed soil
- In Control quadrats, remaining litter was left undisturbed in situ.

Pre-disturbance *A. whibleyana* seedling tagging or translocation

- *A. whibleyana* germinants growing within Control quadrats were left in place, and tagged with a brass tag.
- *A. whibleyana* germinants growing within Disturbed quadrats were translocated to a nearby spot beneath the same adult plant, away from treatment quadrats, and tagged with a brass tag.

Seed collection and viability

- A total of 6,600 seeds were collected from 12 mature *A whibleyana* shrubs at the Salt Lake stand on 9 January 2019
- X-ray analyses showed 66% of seeds were viable, 14% (7 seeds) appeared to have been predated (one still has a grub inside), and 20% appeared to be damaged due to predation or other developmental problems (J. Guerin pers. comm.).

Power analysis to estimate expected germination responses and optimal number of seeds for seed supplementation experiment

- Literature review to identify all published Acacia germination trials, using Web of Science (search terms: Acacia, germination, seed), and all existing *A. whibleyana* published and grey literature. Estimated expected germination rates based on previous *A whibleyana* and other Acacia germination studies.
    - Control quadrats (no disturbance) – expected up to 3% germination, based on 3.3% germination in untreated *A. whibleyana* seeds incubated in dark on vermiculate at 20 degrees and watered (Jusaitis and Sorensen 1998)
    - Disturbed quadrats – estimated germination response could range from 17–70%, including 66% *A whibleyana* seed viability (SA Seed Centre 2020). Low response from 17%, based on seedcoat nicking (Jusaitis 1998); high response up to 70%, based on seed nicking (Jusaitis and Sorensen 1998)
    - Seed supplementation – expected *A. whibleyana* seedbank from 42–113 seeds/m2, based on 42 seeds/m2 at Quarry stand and 113 seeds/m2 at Salt Lake stand (Jusaitis and Sorensen 1998).
- Power analyses using linear mixed models predicted that addition of 25 seeds would detect statistical differences based on our expected Control (3% germination) and Disturbed (17–70% germination) responses, provided the above assumptions were met.
- To reduce the likelihood of zero inflated data preventing detection of a very weak germination response (<3%) in Control quadrats (no disturbance or seed supplementation), compared with high germination response (>70%) in Disturbed quadrats (disturbed and seed supplemented), we conducted the seed supplementation as a separate experiment.

Experiments 1 and 2: Disturbance treatment

- Carried out on 24th & 25th June 2020
- Soil disturbance was achieved using a 127cc Rotary Hoe
- Trialed using Skittles and M&M chocolates to assess potential distribution of “seeds” within disturbed soil
- Disturbance to an average depth of 6.7 cm (range = 6.5–8 cm) with a single pass of the hoe by wiggling the machine at a consistent rate while pulling it backward over the quadrat
- Disturbed soil was gently pushed back to redistribute it over the quadrat.
- All quadrats, regardless of treatment, were watered within 3 hours of disturbance until all cells were saturated (approx. 2 L per quadrat), but not flooded ,to optimise germination success

Experiments 1 and 2: Water treatment

- Rainfall calculated for each month.
- Supplemental water was applied up to the monthly rainfall average.
- The volume of water required was calculated by subtracting the previous month’s rainfall (recorded by a private landholder a few kilometers from the Salt Lake study site) from the monthly average rainfall.
- Monthly average rainfall was calculated using the midpoint between the average rainfall recorded at Tumby Bay and at Yadnarie weather stations as this most closely represented actual rainfall recorded near the study site (rainfall at the site was consistently higher than Tumby Bay station and lower than Yadnarie).
- When the previous month’s rainfall was lower than average, the difference was applied during the first week of every month from September 2020 onwards.
- Water was applied evenly across the subquadrats using a watering can with a rain nozzle attached.

Experiment 3: Seed supplementation treatment

- All natural seedbank quadrats (Experiments 1 and 2) were paired with seed supplemented quadrats (Experiment 3)
- For each subplot, Whibley seeds were added to the left quadrat (facing inwards to the shrub centre; Figure x). The right hand quadrat had no seed addition.

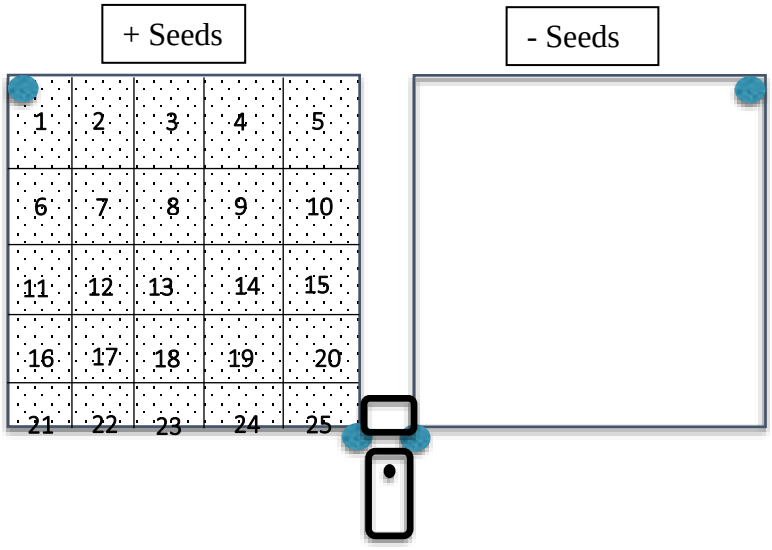


Figure 2 Example of cell numbering for Experiment 3: +Disturbed (L) and Control (– disturbed) quadrats within one subplot. Aqua circle indicates nail.

- Seeds were NOT pre-treated as the goal of this trial was to determine whether mechanical soil disturbance can stimulate, and increase the rate of, germination in *A whibleyana* seeds
- 25 untreated seeds were counted into vials (one per quadrat) to standardize the seed addition treatment and ensure no seeds were wasted
- Seeds were added to **Disturbed +Seed quadrats** (24th & 25th June 2020)
    - 25 seeds were added to two cells in the quadrat (cells 7 and 9; Figure x), half in each, as pre-trials had confirmed that seeds would then be distributed throughout the quadrat by the disturbance treatment (see Disturbance Treatment).
- Seeds were added to **Control –Seed quadrats** (25th June 2020)
    - One seed was placed into a 1cm deep depression made with a nail, in the centre of each cell, and the hole was filled in with loose soil and tamped down lightly to cover the seed.
    - Seeds were buried to reduce predation (birds and invertebrates such as ants) based on burying to 0.5-1cm deep by Jusaitis and Sorensen (1998; see also Clarke and Davison 2001 who reported studies of other acacias showed seedling emergence was higher for seeds buried ~1cm deep).

Appendix 2

GANNT Chart of Project Milestone Completions and Progress