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Tetratheca erubescens Translocation Final Research Report

for Mineral Resources Limited March 2021 to March 2022

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TABLE OF CONTENTS

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EXECUTIVE SUMMARY

Kings Park Science's "*Tetratheca erubescens* Translocation Research Program" commenced in June 2017 and was carried out over five years. The broad objectives of the Research Program were to (i) develop methods to support translocation and restoration of *T. erubescens;* (ii) provide technical and scientific support for a five-year research program of field translocation; and (iii) assess functional attributes within restored / translocated populations to determine their long-term sustainability compared with natural populations. The Research Program quantified ecological, demographic and physiological traits of *Tetratheca erubescens* to inform practical approaches of experimental translocations and to understand key limitations to translocation success.

The Research Program involved over 100 staff, volunteers and students to deliver comprehensive translocation outcomes that have been communicated and promoted in reports, scientific articles, international and national conferences, radio, workshops, and used as a training platform for students. The multi-year program was critical for obtaining research important to identifying and developing techniques and approaches that improve translocation success. This final report is structured in two parts: **Part 1** summarises all the research outcomes of this project (June 2017 – March 2022), and **Part 2** summarises research results completed during March 2021 – March 2022, that has not been previously reported. Detailed information on the research objectives can be found in **Annual Research Reports 1 - 4** (2018-2021).

The Research Program (2017 – 2022) delivered:

i) Seed biology research to inform best practice approaches to the management and use of seeds in propagation and translocation techniques. This objective delivered methods for reliable propagation via seed (80% germination under *ex situ* conditions) and increased the capacity of seeds to germinate in translocation sites (germination of 12.5%). It also found that although germination was possible under relatively low levels of water availability, seedlings of *T. erubescens* require continuous water availability after germination to support emergence.

ii) Translocation research to improve methods for propagule collection, propagation, translocation installation and monitoring. This objective developed the methods to reliably propagate and deliver seedlings and greenstock to translocation sites. Although overall survival of translocated plants was low (3%), due to high mortality rates in the first summer, surviving plants achieved a similar phenological and ecological function (growth, flowering, fruiting periods) compared to plants in natural populations. Translocated plants were, however, unable to support the production of significant quantities of seed and dispersed only a small number into the soil seedbank.

iii) Plant function, habitat and substrate interactions research compared the function of plants in natural and translocated populations to inform translocation management practices. This identified that *T. erubescens* plants have a drought avoidance strategy, with plant ecophysiological function generally maximised during winter and on SW-facing aspects where conditions were not as stressful (17% cooler; 95% wetter than NE-facing aspect). Trends in plant performance across different sites and years support this observation. Where appropriate niches were found in translocated sites, surviving translocated plants developed similar plant function to that of natural plants after two years. Seedling recruitment from both direct seeding and natural soil seedbanks was limited by seedling survival (narrow habitat niche) rather than seedling emergence (broad habitat niche).

The Research Program identified techniques and approaches to be able to implement translocations of *Tetratheca erubescens*, and defined the barriers that need to be overcome to improve outcomes. Translocation achievements have been reviewed nationally (Silcock *et al*. 2019) and low plant survival in these experimental translocations is common place for threatened species translocations. This highlights the need for large propagation and multi-year translocation efforts that continue to seek direction from appropriate research programs. For *T*. *erubescens*, seedling and greenstock survival over the first summer was a key limiting step. There was clear evidence for the need to overcome water limitations in the system (at least within the first year) and to assess additional planting locations in the existing or reconstructed habitat. This approach when integrated with new knowledge on interactions between ecological, demographic and physiological traits identified in this Research Program will likely improve translocation outcomes. From the perspective of how translocated *T*. *erubescens* populations then transition to a self-sustaining population, longer timelines are required to 1) quantify seed input into the seedbank and 2) confirm the recruitment capability for the next generation of plants.

BACKGROUND

Cliffs Asia Pacific Iron Ore (Cliffs) received conditional approval to develop a new mining area at F Deposit in the southern Koolyanobbing Range (Ministerial Statement 1054). The development at F Deposit involves the removal of individual plants of the Declared Rare Flora species *Tetratheca erubescens*. The Ministerial Statement includes a requirement for a program of research and restoration as part of the Stage 1 *Tetratheca erubescens* Offsets Plan. Cliffs originally engaged with the Botanic Gardens and Parks Authority (BGPA, or 'Kings Park Science') and formed a partnership to deliver a translocation research program for *Tetratheca erubescens* that supports the Offsets Plan. In 2018, Mineral Resources Limited took over operation of the F Deposit project area and implementation of the Translocation Research Program (with Kings Park Science, a science program under Biodiversity and Conservation Science in the Department of Biodiversity, Conservation and Attractions).

Tetratheca erubescens occurs in the Koolyanobbing area, in the Coolgardie IBRA (Interim Biogeographic Regionalisation for Australia) bioregion of Western Australia. This species has Threatened flora status under the *Biodiversity Conservation Act* (WA) 2016 with a very narrow distribution associated with a single banded iron formation (BIF) range where it grows in rock fissures on cliff faces. This extreme habitat provides significant challenges for restoration and translocation of populations. Effective, sustainable translocation of individual plants and populations requires understanding of attributes of the species and its habitat, including population processes and interactions with the environment, as well as appropriate propagation and translocation techniques.

This translocation research program aimed to:

- Develop methods to support the translocation of *T. erubescens.*
- Provide technical and scientific support for a five-year research program of field translocations of *T. erubescens*. MRL's objective is to establish a new self-sustaining population of at least 313 mature individuals of *Tetratheca erubescens* on landform that is suitable for the species.
- Assess functional attributes within restored/translocated populations to determine their long-term sustainability through a comparison with natural populations.

This document reports on the outcomes of the scientific approach that:

- Developed practical, effective and sustainable translocation of *Tetratheca erubescens.* This was achieved through understanding and optimising their establishment ecology and environmental requirements.
- Determined how these approaches can be effectively utilised or recreated in restored systems. Thus enabling the long-term persistence of the species and viability of disturbed populations.

The Kings Park Science research program addresses the science required to underpin and inform translocation efforts by MRL. Occurring concurrently was an Offset Plan, derived and agreed upon by MRL and relevant regulatory authorities. Although Kings Park Science was not involved in developing the Offset Plan and associated milestones *per se,* it was understood that the Kings Park Science program will assist MRL in the science investigations relevant to the Offset Plan.

RESEARCH PROGRAM

The translocation of species whose habitat is confined to narrow cracks in rock outcrops is a challenge that significantly exceeds the complexities of a normal translocation program. The general principles of effective and sustainable translocation (Commander *et al*. 2018) include:

1. Understanding a) the interactions between plants and the environment in their natural habitat and b) the ecological, genetic and demographic population processes that enable self-sustained growth and persistence of natural populations.
2. Selecting, modifying or creating an appropriate translocation site given 1a.
3. Selecting plant material and developing translocation techniques that will enable the survival of the number of individuals required given likely attrition rates, with the appropriate level of population genetic diversity and representation given 1b.
4. Implementing, maintaining and monitoring the translocation.
5. Typically, translocation research and translocation programs involve an iterative learning/adaptive management approach and a scaling-up from experimental to implementation phases.

We have adopted these principles and executed a research program through investigation in three key disciplines: seed biology and enhancement (Program 1); translocation and demographic studies (Program 2) and plant-substrate interactions (Program 3).

PART 1: RESEARCH OUTCOMES 2017-2022

Program 1. Seed biology

1.1 *Dormancy and germination.*

1.1.1 Assess the sensitivity of seeds to constant and alternating incubation temperatures under differing light regimes.

Research outcomes:

- Understanding the sensitivity of seeds to constant and alternating incubation temperatures under differing light regimes is important for determining the germination niche, in terms of their thermal requirements across seasons and in relation to seed burial depth. Seeds were incubated across a temperature gradient between 5°C and 40°C, and under 12/12 hour alternating temperature regimes within the germination range for constant temperatures.
- Optimal temperature for germination was between 15-20°C (even after breaking dormancy through warm stratification, see Section 1.1.3). These conditions coincide with those of autumn, winter and spring, rather than summer. Maximum germination under optimal temperature conditions were 75-80%. At temperatures of 25°C and hotter, germination did not occur.
- Alternating temperatures (e.g. 20/10°C, or 25/15°C) did not support germination. The requirement for constant temperatures for germination is believed to be a depth sensing mechanism to changes of water availability to improve seedling survival. Alternating temperature conditions signal shallow burial in soil or rock where greater variation in water availability was experienced.
- Under optimal temperature conditions, there was no difference between alternating light/dark and constant dark conditions for seed germination. For propagation and field scenarios, light conditions are therefore not inhibitory for germination.
- Details of research are in Annual Research Reports 1-3 (Elliott *et al.* 2018; 2019; 2020).
- **Conclusion:** For nursery propagation, seedling production can be optimised by germinating seeds under 15-20°C temperatures. The research also highlights an ecological window for recruitment and monitoring programs that should target winter and spring periods to quantify recruitment events.

1.1.2 Profile the sensitivity of seeds to water stress during germination.

Research outcomes:

- By profiling the sensitivity of seeds to water stress, we gain a critical insight into the minimum thresholds of water availability for germination. This knowledge informs the rainfall requirements for seeds to germinate, or can be used to determine field manipulation strategies (e.g. irrigation) to support seed germination and seedling establishment. Seeds were incubated along a water stress gradient controlled by different polyethylene glycol solutions, ranging between freely available water (e.g. 0 MPa) and the permanent plant wilting point (-1.5 MPa).

- Seeds require at least 14 days of optimal water availability (0 to -0.25 MPa) for germination. This moisture window represents continued rainfall periods that saturate soil profiles to field capacity. Coupled with cooler temperature requirements, autumn, winter and spring rainfall is believed to be critical to the germination of seeds.
- At higher water stress conditions (close to the permanent plant wilting point e.g. -1.0 to -1.5 MPa), germination capacity decreased and seeds took >24 days to germinate, demonstrating the capacity for seeds to germinate under low water availability.
- Germination sensitivity to water stress changed between dormant and non-dormant seed batches. Stratifying seeds (as outlined in Elliott *et al.* 2019), and pre-treating with KAR_1 increased germination (its range into water stress; germination speed).
- Details of research are in Annual Research Report 3 (Elliott *et al.* 2020).
- **Conclusion:** We have identified that seed of *T. erubescens* restrict their germination to cooler temperature conditions, and have the capacity to germinate under low soil water potentials. Although germination was possible under relatively low levels of water availability, seedlings require continuous water availability after germination to support recruitment. The outcomes of this research explain recruitment pulses observed in the natural population, whereby seedling emergence were observed during winter and spring, yet the ongoing survival of seedlings was significantly impaired, as higher temperatures and long durations between rainfall failed to support the requirements for seedling survival.

1.1.3 Identify the optimal conditions required for promoting dormancy loss focussing on after-ripening, wet/dry cycling and stratification.

Research outcomes:

- Fresh seeds of *T. erubescens* are dormant at dispersal: seeds will not germinate under conditions considered optimal until dormancy is alleviated. Identifying optimal conditions for promoting dormancy loss are key to enhancing *ex situ* and *in situ* management initiatives for *T. erubescens* (e.g. for propagation of seedlings, or pre-treatment of seeds prior to translocation).
- Three treatments were trialled to alleviate dormancy: i) dry after-ripening, whereby seeds were incubated under controlled conditions of 50% relative humidity; ii) stratification, whereby seeds were incubated under controlled warm (e.g. constant 30°C) wet conditions, and iii) wet/dry cycling, whereby seeds were exposed to a combination of cycling through after-ripening and stratification treatments.
- Stratification for six weeks was the optimal treatment to alleviate seed dormancy (germination up to 70%).
- Stratified seeds demonstrate an increased capacity for seeds to germinate at lower levels of water availability.
- Details of research are in Annual Research Reports 1 and 2 (Elliott *et al.* 2018; 2019).
- **Conclusion:** Applying a stratification method to alleviate seed dormancy in *T. erubescens* increases seed germination for seedling production. For field trials,

stratification of seeds should be conducted in order to maximise recruitment probabilities in the field.

1.1.4 Define the role of germination stimulants in promoting germination.

Research outcomes:

- After seed dormancy is alleviated, applying germination stimulants can further enhance the capacity of seeds to germinate, by increasing germination speed and maximum germination performance. Initial investigations trialled different germination stimulants such as gibberellic acid (GA) and karrikinolide (KAR_1). While GA is a growth promoter, KAR_1 is a molecule synthesised from smoke, and has the capacity to stimulate germination in a number of species from fire-prone ecosystems. Preliminary trials, as described in Elliott *et al*. 2017, have demonstrated increased germination performance in seeds of *T. erubescens* following treatment with KAR_1 compared to GA. As such, in each of the temperature, water stress and dormancy alleviation experiments as outlined in Sections 1.1.1-1.1.3, seeds were treated with and without KAR_1, by using a 24h seed soak prior to incubation.
- KAR_1 significantly improves germination by 10-15% at temperatures between 10-20°C in fresh seeds and during dormancy loss following stratification, after-ripening and wet/dry cycling, but does not increase the germination range into cooler or hotter temperatures.
- KAR_1 was found to increase the capacity for seeds to germinate into water stress by at least 10%.
- Details of research are in Annual Research Reports 1 and 2 (Elliott *et al.* 2018; 2019).
- **Conclusion:** Pre-treating seeds with KAR_1 prior to sowing into the field could increase germination rates after sufficient rainfall.

1.2 *Seed enhancement to improve seedling establishment.*

1.2.1 Assess the potential of seed priming to enhance germination and seedling establishment in the field.

Research outcomes:

- For many agricultural species, primed seeds generally have increased germination rates than non-primed seeds. Two methodologies were tested on dormancy alleviated seeds (through a 6-week stratification period, as determined in Section 1.1.1; see Elliott *et al*. 2018); using 1) hydropriming and 2) osmopriming methodologies.
- Osmopriming after 8 days demonstrated highest germination of up to 80% and an increase in germination speed (16.6 days to initial germination) when compared to non-primed stratified seeds (19.9 days to initial germination).
- While priming increases maximum germination and speed responses, there was no apparent benefit under water stress conditions, which is a limiting factor in the field.

- The current tested priming-methodology is also likely to induce conditional dormancy (e.g. high germination under narrow optimal conditions) for *T. erubescens* seeds. Conditional dormancy could be an unfavourable condition that reduces seed germination probabilities.
- Details of research are in Annual Research Report 4 (Elliott *et al.* 2021).
- **Conclusion:** Seed priming showed some capacity to improve germination, however, the lack of benefit of seed priming under water stress and the likely outcome of induced conditional dormancy, means we do not recommend this treatment for field sowing in translocation trials.

1.2.2 Investigate seed coating and seed pelleting approaches for improving seed germination and establishment in the field.

Research outcomes:

- As we have developed an in-depth understanding of how the internal seed state can be modified through addressing seed dormancy, or by testing priming methodologies, a pelleting trial was conducted to investigate the feasibility of manipulating the direct external surrounding environment of the seed in order to maximise seed germination and emergence following rainfall in the field.
- In summary, the pelleting techniques were not suitable for *T. erubescens*, due to loss of seeds during the mechanical process of producing small pellets and the impracticality of finding suitably sized cracks to accommodate a larger pellet in the field.
- A 'slurry' matrix was considered more practical to employ in the field rather than pellets, as its application into an artificial propagation structure was successful.
- Emergence was observed from the 'slurry' soil matrix, but the response was very low and the enhanced treatment performed the same as the control treatment.
- The tested 'slurry' soil matrix did not improve emergence responses of *T. erubescens*.
- Details of research are in Annual Research Reports 2-4 (Elliott *et al.* 2019; 2020; 2021).
- **Conclusion:** The seed enhancement approaches tested here did not improve seed germination or emergence under controlled (*ex situ*) conditions and require further research to determine their applicability.

Program 2. Translocation and monitoring

2.1 *Optimising translocation approaches*

2.1.1 Assess the effectiveness of treated *in situ* sown seeds for undertaking translocations.

Research outcomes:

- Seeds are a critical source for translocation programs. Optimising their effectiveness for use in translocations is essential to maximising seedling establishment and minimising seed waste.
- We assessed the germination response of treated seeds in the field through burial trials and direct seeding trials along cracks or artificial drill holes across translocation sites (see Part 2: Table 2.1b). Seed burial trials provide a means to quantify the persistence of seeds under field conditions. Understanding seed persistence is critical for understanding population sustainability, as this process determines the ability of a species to survive in the soil seedbank.
- Seeds transition from a dormant to a non-dormant state within a year of burial and pre-treated seed have the highest transition (100% transition for treated seed; 80% transition for untreated seed after 485 days of burial).
- Seed germination pulses mainly occur during winter and pre-treated seeds have a faster germination pulse after sowing (12.5% compared to 2.5% after 56 days).
- Irrigation supported germination after sowing (8% irrigated compared to 2.5% non-irrigated after 70 days), however the transition from the germinated seed to the emerged and surviving seedling was a bottleneck.
- *In situ* sown seeds resulted in low seedling emergence (overall 2.5%) and survival (overall <1%) at most translocation sites (except T23).
- Mortality of seedlings was highest after their 1st summer (>96%).
- Seeds can persist for at least three years in the soil seedbank, with modelled declines of 50% viability (p50) of at least 1170 days.
- The low germination and high survival responses of *T. erubescens*, reflects a favourable strategy for plant recruitment in a water-limited ecosystem, as seed losses are minimised over time. Comparing recruitment strategies among other BIF species illustrated substantial differences, highlighting poorer seed persistence and higher mortality rates of seedlings after emergence in co-occurring species.
- **Conclusion:** Translocations can be undertaken with pre-treated seeds sown *in situ* into drill holes or large cracks because seedlings will emerge under the appropriate environmental conditions, and seeds will persist in the environment for at least 1170 days, ensuring opportunities to recruit in the future.

2.1.2 Assess the feasibility of using greenstock derived from different sources (seeds, vs cuttings) for establishing new plants *in situ*.

Research outcomes:

- The use of greenstock allows for flexibility in establishing translocations, particularly if there is a low quantity of seed or the seed is difficult to germinate and/or establish as seedlings.
- Greenstock can be derived from seeds or cuttings to provide translocations with more developed material that can be planted, rather than directly seeded at installation. However, understanding the survival differences between the two types of material is important for improving translocation success and maximising the use of limited source materials from the natural population of *T. erubescens*.
- There were similar levels of survival for seedling (4.2%) and cutting (3.4%) derived greenstock at the early stages of the translocation (2 years; Part 1: Table 2.2.1b).
- Details in Annual Research Reports 3 and 4 (Elliott *et al.* 2020; 2021).
- **Conclusion:** *Tetratheca erubescens* greenstock derived from seeds or cuttings can be used to establish plants in translocation sites *in situ*.

2.1.3 Determine the environmental requirements (crack attributes, aspect, temperature and moisture) for establishing plants *in situ*.

Research outcomes:

- Understanding the environmental requirements for plant establishment *in situ* is key to refining and improving translocation procedures and techniques because these variables directly affect the responses of plants to changing conditions or their planting location.
- The environmental requirements for establishing plants *in situ* were determined by monitoring soil microclimate using high resolution soil moisture and temperature loggers, and characterising the habitat (substrate, position, vegetation) of the planting locations (one-off installation in 2017).
- Peak soil temperatures were up to 69°C in summer 2017/2018, and <60°C in 2018/2019, 2019/2020, and 2020/2021 summers.
- The hot summers in 2019/2020 and 2020/2021 were matched by prolonged periods without moisture recharge from rainfall.
- Baseline (*ex situ*) plant function responses to drought declined after three days and plants significantly deteriorated after ten days, indicating a functional threshold to reductions in soil moisture availability.
- The environmental requirements of greenstock planting locations that would more likely support survival appear to be a steeper, continuous local slope or a non-continuous local slope (a right-angled position); a large immediate local catchment area; and a smaller width to the planting area (or shelf).
- Crack attributes (around planting location) or aspect did not appear to be key characteristics that indicated improved greenstock survival.

- There were too few seedlings established from *in situ* sown seed to characterise their environmental requirements effectively.
- Details of research are in Annual Research Reports 2-4 (Elliott *et al.* 2019; 2020; 2021) and Part 2 of this report.
- **Conclusion:** Changing conditions (temperature and soil moisture) and planting location (habitat characteristics) strongly affect plant responses and these specific environmental requirements need to be considered when selecting translocation sites, planting locations and exploring potential field amendments (e.g. irrigation) to improve plant establishment. These research outcomes provide valuable information for the targeted placement of new planting and sowing locations at existing translocation sites (or new translocation sites) to increase survival outcomes under future installation opportunities.

2.1.4 Compare the responses of plants when placed *in situ* into different locations including within, adjacent and outside of known *T. erubescens* populations, and into artificial sites created as a consequence of mining.

Research outcomes:

- Understanding the responses of plants that are translocated into sites with different contexts is important for quantifying the suitability of the sites that were selected for translocation. If one particular location performs better than the others, determine why that this response was observed as this will inform potential adaptive management activities (e.g. select alternate sites or adjust *in situ* practices for certain locations).
- Plant responses (i.e. survival) between adjacent and outside known *T. erubescens* populations were similar among most translocation sites, except one located adjacent to natural populations on the northern side of the ridge (T23), where survival was noticeably higher.
- Approval to test plant responses within *T. erubescens* populations was not granted for experimental translocation.
- Only one artificial site was tested and was offline after a year because of landform instability associated with mining activities, therefore, more options of testing artificial substrates are required to address feasibility.
- Details are in Annual Research Reports 1-4 (Elliott *et al*. 2018; 2019; 2020; 2021).
- **Conclusion:** Based on the limited range of translocation sites adjacent to- or outside of- known *T. erubescens* populations that were tested and the mixed response of the adjacent sites, it is inconclusive as whether one may or may not be better as a translocation site and therefore, does not exclude any sites for testing for use as translocation sites.

2.2 *Survival, growth and reproduction in restored and natural populations.*

Research outcomes:

- Understanding demographic processes and their variability in relation to environmental conditions, seasonal patterns and life stages is key baseline information for any translocation project. The quantification of plant growth, survival, flowering, seed production, recruitment and age of maturity of plants in the natural population 1) identifies the existence and importance of demographic events (e.g. recruitment, mortality) for population dynamics; and 2) provides the reference point to which the performance or response of translocated individuals can be compared against.
- Baseline data on the growth, survival, flowering and seed production of seedlings, juveniles and mature plants is summarised in Table 2.2.1a and of greenstock and directly sown seeds from the four experimental translocations are summarised in Table 2.2.1b and Figure 2.2.1. Annual variation in parameters and variation between the southern and northern side of the range were observed, which indicated climate and environmental conditions were highly influential.
- These tables provide brief and condensed data for each life stage or propagule type of *T. erubescens* (see Annual Research Reports 1-4 (2018-2021) and Part 2 of this report, for information on the various treatments implemented each year). Further research outcomes of the performance comparison between natural and translocated plants are detailed in Part 2: Section 2.2.4 and Table 2.2.4a of this report.
- **Conclusion:** Four years of monitoring has been critical to capture annual variation in responses and has been of suitable length to develop a baseline for understanding the response of each life stage, propagule type, and the outcomes of translocation methods and certain ecological parameters. However, monitoring should continue because it was too short to accurately capture life stage transitions (e.g. seedling to juvenile) or functional transitions (e.g. seed production for seedlings) or threshold transition potential (e.g. seasonal or extreme changes to health) and poorly represents the impact of the longer-term cycling of climate conditions (e.g. La Niña vs El Niño).

2.2.1 Develop baseline data on the growth, survival, flowering and seed production of seedlings, juveniles and mature plants.

Natural population

Research outcomes:

- Survival was poorest at the seedling life stage and highest at the mature adult life stage, indicating that seedling establishment after seedling emergence is a likely key bottleneck to successful recruitment.
- Age to first flowering occurred from three years, with increasing numbers of juveniles observed to flower and fruit (full size and dehisced stages) in successive years. There was much variation in the age to which these transitions occurred, indicating that the environment (location and climate) was important in determining development of individuals.

- Flowering occurred in >90% of the monitored mature plants (per year) and seed production was an average of 5-11 seed/stem for mature plants.
- Plant growth was highest for the seedling life stage (90% of existing plant biomass), which was expected due to their comparatively small size. For adult plants, the addition of new stems during the growing season was 57% of the existing plant biomass.
- Plant condition of seedlings and juveniles was similar between spring and summer and this was a smaller decline in condition (<1%) than mature plants, which showed the greatest decline in condition during summer (3%). This indicated that summer environmental conditions were less likely to support much of the biomass gained during the growing season for mature plants (i.e. senesced over summer), particularly as the individual variation in this decline could reach up to 50%, unlike the younger life stages.
- **Conclusion:** The dynamics and demography of *T. erubescens* populations indicate that each life stage had certain strategies to cope with a water-limited environment and their sensitivity to environmental stress varied. These include, a pulse recruitment strategy, seedlings that are highly sensitive to environmental stress (i.e. summer), adults that are less sensitive to environmental stress, seasonal responses in above ground biomass or plant health to changing conditions that manages plant survival, and a reproductive capacity that takes advantage of appropriate environmental conditions (i.e. opportunistic).
- The formation of this baseline data on natural population parameters, which signify functional capacity and performance under certain environmental conditions provides measures of direct comparison that can be made against the performance of translocations, as they have been used for the 2017-2020 translocations or for ongoing translocation monitoring or future translocations.

Translocated populations

Research outcomes:

- Survival of greenstock and emerged seedlings were similar (relative to the numbers observed), indicating that either propagule source can be used in translocations with similar survival performances.
- Despite survival being the poorest after the first summer (3.2-5.4%), survival was higher in the subsequent summers (25-100%; Part 1: Figure 2.2.1; limited data on seedling derived greenstock). This indicated that survival in translocation sites was highly dependent on the conditions experienced in the first summer post-planting or emerging, and less so on surviving the following summers.
- Flowering and fruit production occurred immediately (spring following planting) for cutting derived greenstock and at two years of age for seedling derived greenstock. Seeds were only opportunistically collected, as greenstock plants were too small-fragile to passively collect seed, therefore production quantities could not be accurately assessed. None of the *in situ* emerged seedlings in translocations flowered after three years, which was different to minimum age of seedlings in the natural population. It is unclear whether this difference was within the variation observed for natural seedlings (i.e. not all flowered at that age) or be due to differences in the environment of the

translocation site because the monitoring term was short and there were very few seedlings to monitor.

- Plant growth was high for the greenstock, which was possibly due to their comparatively small size at planting, with seedling derived greenstock having the highest increase of plant biomass (160%). Interestingly, this increase in relative plant biomass was within the range observed for naturally recruited seedlings. This indicated that the growth of greenstock responded to the environment in a similar manner as a newly emerged seedling did in the natural population.
- Plant condition of seedling and cutting derived greenstock had a greater decline in summer than spring, which was more within the ranges observed for the mature adult plants in the natural population. This also indicated that summer environmental conditions were unable to support much of the biomass gained during the growing season for greenstock (more so those that were cutting derived) at the translocation sites (i.e. senesced over summer).
- **Conclusion:** The initial dynamics of translocated populations indicated that each stage of the process had certain responses to environmental stress that showed strong sensitivities that led to large mortality events. Recruitment from direct seeding and the survival of those seedlings were as sensitive to environmental stress as the natural population, but the performance of greenstock was closer to that of a seedling than an adult in the natural population.
- The formation of this baseline data from the translocated population, which can be compared to the natural population provides key information for improving survival at the more sensitive stages of a developing translocated population (i.e. greenstock or seedling establishment during the first summer).

Table 2.2.1a. Baseline data on the growth, survival, flowering and seed production of the three life stages (mature plant, juvenile, seedling) observed in the natural population. The overall average for the propagule is presented, with the range in variation presented in parenthesis, which captures annual variation, and differences between plants on the northern and southern aspects. See Annual Reports 1-4 (2018-2021) or Part 2 of this report.

	Mature	**Juvenile**	**Seedling**
Parameters			
Monitoring period	4 years	3 years	3 years
No. plants monitored	182	17	21-252 per year
Plant age	Unknown	Unknown	Known
Age of reproduction	-	Unknown	>3 years
Plants reproductive at start of study	Yes	Some	No
Percentage survived	>98.9% per year	94.1% per year	5.5 - 20.5% after 1st summer
Reproductive phenology	• Begins in May • Peaks in October (unless limited rainfall) • Finishes in January • Can be opportunistic		
Percentage reproductive	>90% per year	23.5% at 1st spring	22.2% at 3 years-of-age
		56.3% at 3rd spring	
Percentage produced fruit (of those reproductive)	>91.6% per year	>67% per year	1 of 2 of the reproductive seedlings
Average seed production	1-2 seed/fruit[^] 5-11 seed/stem[&]	Unknown*	Unknown*
Average growth (% new stems per year)	57.0% (38.1 – 71%)	62.3% (40.0 – 90.0%)	90.0% (50 – 130%)
Average plant condition (% plant brown)	26.3% spring (21.0 – 35.6%) 23.6% summer (18.3 – 54.5%)	10.4% spring (6.3 – 13.6%) 17.6% summer (7.9 – 15.9%)	10.4% spring (4.8 – 12.2%) 11% summer (5.6 – 14.1%)

Seedling = monitored post-emergence.
Juvenile = untagged (i.e. not in previous surveys) small plants & juvenile foliage (leaves on all stems).
Mature = tagged (present in previous surveys) plants that were reproductive.
* Plants too small-fragile to passively collect potential seed by attaching an organza bag.
[^] Occasionally 3-4 seed/fruit were observed.
[&] Stems: ranged in length depending on the size of the plant, but most stems were ~30cm long.

Table 2.2.1b. Baseline data on the growth, survival, flowering and seed production of cutting or seedling derived greenstock and seedlings that emerged from directly sown seeds, in the translocated sites. The overall average for the propagule is presented, with the range in variation presented in parenthesis, which captures annual variation, and differences between plants on the northern and southern aspects. See Annual Reports 1-4 (2018-2021) or Part 2 of this report.

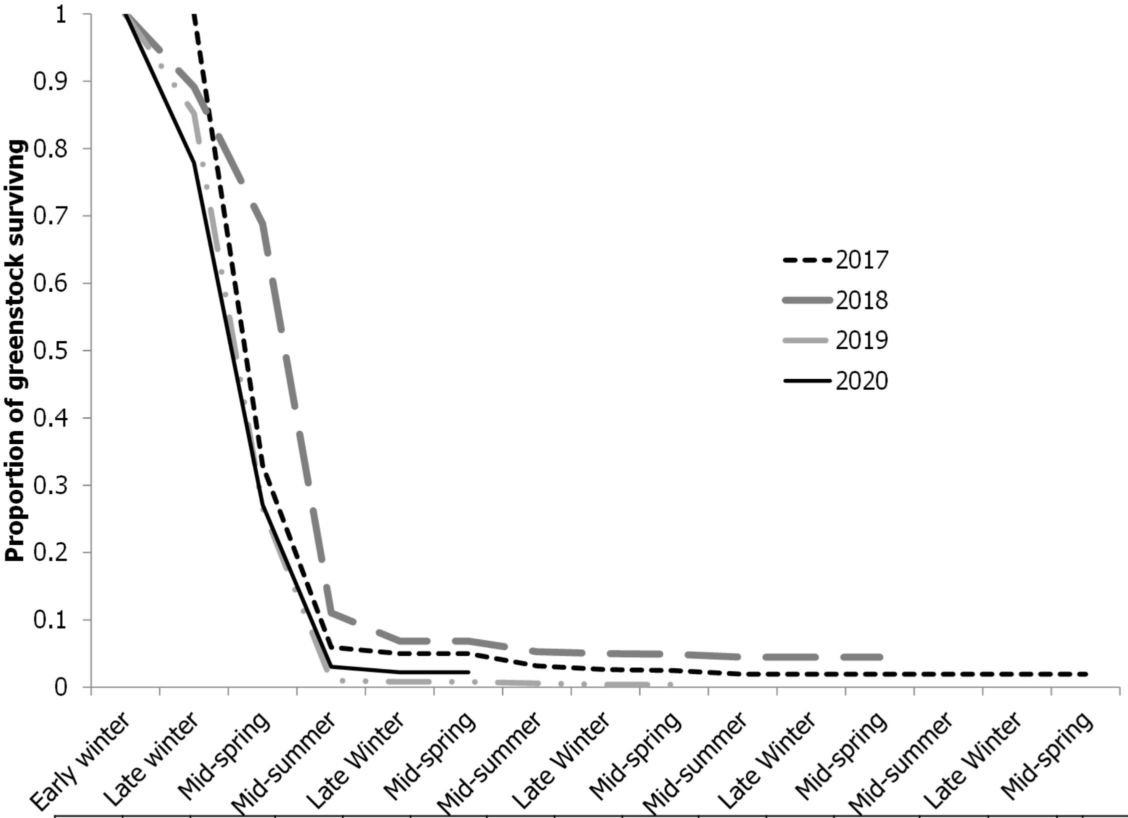
	Greenstock		**Seed**
Parameters	Cutting	Seedling	Direct sowing
Monitoring period	>4 years	2 years	3 years
No. plants monitored	2,546	546[ϕ]	17-80 per year
Plant age	Un/Known	Known	Known
Age of reproduction	1st spring	2 years	Unknown
Plants reproductive at start of study	Yes	No	No
Percentage survived (overall at end of 2021)	3.4%	4.2%	0.8%
after 1St summer	5.4% (1.2 – 11.4%)	4.2% (2.1 – 5.3%)	3.2%
Of the survivors after 2nd summer	49.8% (25.0 – 90.0%)	50% (0 – 100%)	25.0%
Of the survivors after 3rd summer	78.1% (50.0 – 92.3%)	-	100%
Of the survivors after 4th summer	100%	-	-
Percentage reproductive (of the total alive per year)	35.4% (10.1 – 58.8%)	17.4%[€]	0%[§]
Percentage produced fruit (of those reproductive)	23.4% (1.3 – 53%)	0%	-
Average seed production	Unknown*	Unknown*	-
Average growth (% new stems per year)	96.5% (39 – 168%)	160% (0-550%)	-
Average plant condition (% plant brown)	15.5% spring (4.6 – 25.4%) 27.9% summer (20.9 – 42.1%)	11.8% spring 37.3% summer (2.1 – 21.5%)	-

[ϕ] Limited number of seedling derived greenstock available to assess for most parameters (*n* = 22).

[€] Seedling greenstock flowered one-year post-planting or ~two years of age (*n* = 2).

* Plants too small-fragile to passively collect potential seed by attaching an organza bag. However, opportunistic collection of a few mature fruits (2019 = 10; 2020 = 2; 2021 = 3) found seed present (2019 = 16; 2020 = 0; 2021 = 5).

[§] Only one seedling survived multiple summers and therefore we could not gather data on the remaining parameters.



29%	29%	85%*	3%	48%	45%	31%	78%	45%*	3%*	5%*	12%*	5%	21%		2017
3%	48%	45%	31%	78%	45%*	3%*	5%*	12%*	5%	21%					2018
31%	78%	45%*	3%*	5%*	12%*	5%	21%								2019
3%*	5%*	12%*	5%	21%							*Late rains				2020

Figure 2.2.1. Overall survival of greenstock planted (2017-2020 translocations) from installation through to last monitoring in mid-spring of 2021. Data represents five translocation sites (T6, T18, T21, T23 and T24) in a combined overall survival rate. The table presents the amount of above (blue) or below (red) average rainfall for that specific period or season (BOM, 2021).

2.2.2 Develop understanding of the importance of spatiotemporal environmental factors that drive variation in these population parameters.

Research outcomes:

- Knowledge of how plant population parameters vary in response to spatiotemporal environmental factors is important for understanding the influences of annual variation and environmental effects on the ecological responses of populations (e.g. reproduction, recruitment).
- Annual variation in winter rainfall may strongly influence the size of the recruitment pulse, but the onset of summer strongly affects the survival of these seedlings in their first year in most situations, regardless of any variation in summer conditions.
- Similarly, the patterns of plant growth, health (condition) and reproduction are likely influenced by climate and the environment (e.g. aspect) and cycle through low

production (hotter or drier) and high production (cooler or wetter) phases depending on the conditions of the environment, with annual variation in these responses detected.
- The specific location for recruitment appeared to be more dependent on the niche being able to support the requirements for the establishment of a seedling (narrower survival niche of cracks) rather than the emergence of a seedling (broader emergence niche of cracks or on the ground).
- **Conclusion:** Spatiotemporal environmental factors are indeed critical in driving the variation in these population parameters (seedling establishment a primary factor) and need to be continually considered when assessing performance measures in both the natural and translocated populations. In this semi-arid environment, it is a dependence on these factors being favourable for plant recruitment, establishment, survival and reproduction that will strongly influence population dynamics, and for translocations, need to be overcome to improve success.

2.2.3 Model the dynamics of *T. erubescens* populations to increase understanding of parameters such as expected longevity and time to maturity.

Research outcomes:

- An expanded conceptual model linking the processes of developing a translocated population to the life cycle of a natural population of *T. erubescens*, demonstrated that the production and incorporation of seeds into the system was the key step to establishing a self-sustainable population.
- The life stage population model analyses predicted a decline in population growth rates of the translocated population and had mixed predictions (decline or growth) of population growth of the natural populations. This is understandable considering the strong annual variation in all the baseline parameters measured (see Part 1: Table 2.2.1a and 2.2.1b).
- The strength of these population model analyses can only be improved with having data on additional translocation responses (i.e. new or improved translocation installations) and longer monitoring lengths (i.e. accurately capture transitions) incorporated into the models.
- Details of modelling findings are in Part 2 of this report (Elliott *et al.* 2022).
- **Conclusion:** Future translocation efforts and research needs to focus on increasing the size of the theoretical link between a translocated population and the life cycle of a natural population by overcoming the survival bottleneck of the translocated population so reproductive maturity is reached and the translocation can successfully transition it to a functioning natural population.

2.2.4 Compare performance of plants (growth, survival, flowering and seed production) in natural and translocated sites.

Research outcomes:

- Quantifying the ecological performance of plants in natural populations provides a point of reference or baseline for comparing the performance of translocated populations and indicates what components of translocated populations that are different or more sensitive and that may instigate further management actions to improve outcomes.
- Survival outcomes for newly translocated greenstock (1.2-11.4%) or just recruited seedlings (3.2-20.5%) were similar – survive the first summer and have a higher probability of surviving in the longer term. Whereas, survival of mature plants in the natural population was very high (99.4%). The difference in survival was likely due to their stage of life, with adult plants in the natural population at a stable phase (i.e. already established), while seedling and greenstock plants are transitioning between phases (i.e. vulnerable pre-establishment stages as emerged seedling or newly planted greenstock, to older and more mature stages). This indicated that location was critical to successful translocation and recruitment in this habitat.
- Growth and plant condition patterns were similar among natural and translocated sites (same winter growing season and decline in plant biomass over spring/summer). However, greenstock and seedlings had the capacity to put on many more stems than the mature plants, that was relative to their smaller size. Interestingly, the summer decline in greenstock condition was closer to that of the mature plants (i.e. greater decline in biomass) compared to the natural seedlings.
- Greenstock flowered and fruited, indicating a reproductive capacity that was able to be detected and visited by pollinators at translocated sites. Long-term studies need to determine seed production baselines for greenstock, and determine the age and reproductive capacity of plants established from directly sown seed.
- Disturbance events (e.g. herbivory, substrate movement) negatively impacted the health and survival of plants at translocated and natural populations.
- Comparisons between natural and translocated plants are in Part 2 (Table 2.2.4a).
- **Conclusion:** Translocations performed differently to natural populations in most quantitative aspects (e.g. overall survival of 99% for natural plants compared to 2.8% for planted greenstock) but not all (e.g. similar condition in summer, <55% of plant brown). More importantly, the phenological and ecological function of translocations responded to environmental factors at the same time as the natural population (e.g. timing of flowering, fruiting, growth, recruitment, senescence and mortality) indicating a positive development for the possible long-term connectivity (e.g. pollen movement, as flowering at the same time) of translocated population to natural populations.
- The formation of these baselines of functional capacity and performance of translocated and natural population parameters allow direct comparisons to be made and improvements on best practice to be implemented. Translocation installation and management strategies should consider improving soil moisture availability (guided by Section 3.1: irrigation, shading) or targeting more suitable planting locations (guided by Section 2.1.3: a large immediate local catchment area). This will improve the trajectory of a translocated population by increasing the number of reproductive plants, and move it closer towards the establishment of a self-sustainable population (guided by Section 2.2.3).

Program 3. Plant function, habitat and substrate interactions

3.1 *Plant function, condition and water usage*

3.1.1 Develop baseline data on the physiology and function of *T. erubescens* plants at seedling, juvenile and adult stages in natural populations.

Research outcomes:

- Knowledge of how each life stage performs physiologically is key to understanding environmental effects on the performance, productivity and survival attributes or sensitivities of each life stage. Measurements in the field of chlorophyll fluorescence (a measure for photosynthetic activity used to assess levels of plant stress), plant condition (a relative measure of plant health used to assess the response of leaf biomass to the environment - stay alive or die), and drought responses by withholding water under glasshouse conditions were conducted to provide baseline data on the physiology and function of plants at different stages.
- Under glasshouse conditions, investigation of drought responses indicated that plants significantly decline at soil water potentials of –0.91 to –1.34 MPa. In contrast, data from field surveys indicate adult plants decrease in plant functioning at –31 MPa (approximately 94% drier conditions), suggesting establishing seedlings to be more sensitive to water stress than the adult plants. Additionally, withholding water for 13 days resulted in 100% mortality of the seedlings, whilst adult plants in the field demonstrate the capacity to survive through prolonged periods without rainfall. A possible explanation for the higher survival in adult plants is likely due to rooting strategies that enable access to moisture within the rock strata (see Section 3.1.3).
- From field surveys, ecophysiological performance (chlorophyll Fv/Fm) was similar between juvenile and adult plants in the natural population throughout the study.
- Details are in Annual Research Reports 2-4 (Elliott *et al.* 2019; 2020; 2021).
- **Conclusion:** The seedling stage was shown to be the most sensitive to drought, whilst juvenile and adult plants appear to be more tolerant. The high sensitivity to drought underpins the low survival rates observed in seedlings across natural sites after the first summer (5-20.5%, Part 1: Table 2.2.1a). Given the increased sensitivity to drought during the seedling stage, future efforts need to focus on improving soil moisture availability (i.e. irrigation) to increase survival.

3.1.2 Assess the impact of spatiotemporal variation in the environment (years, seasons, sites, habitat characteristics) on plant function

Research outcomes:

- Knowledge of how plants perform physiologically is key to understanding environmental effects on plant performance, productivity and survival. Measurements of stomatal conductance – a measure of stomatal opening in plant leaves which can be used an indicator for plant water status; chlorophyll fluorescence – a measure for photosynthetic activity which can be used to assess levels of plant stress, and were conducted across 13 monitoring periods between summer 2018 and summer 2021 to determine spatiotemporal variation in plant performance. Ecophysiological performance of *T. erubescens* was also measured against two co-occurring species (*Banksia arborea* and *Eremophila decipiens*).

- Variation in ecophysiological performance, based on stomatal conductance, are strongly driven by different seasons across years and differences between species. Winter represents the highest periods for ecophysiological functioning across all species, with decreases of 33-51% as the seasons transition into summer periods. Aspect can challenge plant performance, with NE-facing plots having on average a 20-30% decrease in function compared to SW-facing plots. *T. erubescens* and *B. arborea* generally perform poorer on NE-facing plots, whilst in *E. decipiens,* ecophysiological functioning was similar in NE and SW plots.
- Details of research are in Annual Research Reports 1-4 (Elliott *et al.* 2018; 2019; 2020; 2021) and Part 2 of this report.
- **Conclusion:** By measuring spatiotemporal variation in plant performance, we have identified sites on the Koolyanobbing Range that show increased plant stress. Additionally, the patterns measured over time and space provide a critical baseline information underpinning plant and population performance that can be used to inform best practice translocation actions.

3.1.3 Identify the ecophysiological strategies employed by plants that enable them to survive and grow in rock fissures in a semi-arid environment

Research outcomes:

- Identifying the likely ecophysiological strategy employed by *T. erubescens* that enables plants to survive in banded ironstone habitat provides information on how plants are utilising their environment to access moisture and maintain plant function. Exploration of the internal characteristics of rock strata (root extent of *T. erubescens*; water availability) and understanding variation in ecophysiological function (seasonal) will provide evidence for this identification of survival strategy and inform on whether translocation sites can support this strategy.
- Genetic assessment determined that *T. erubescens* roots occurred in the rock profile to a depth of 400cm from the crest, indicating that the plants could exploit the cracks, fissures and cavities of the rock strata to locate potential pockets of accessible moisture.
- Analysis of soil samples at different depths into the rock, and around exposed roots indicated moisture available between approximately 0.15 and 0.32 g/g (gravimetric water content), which was traced back to a rainfall event that had occurred approximately three weeks prior to the rock excavation. This result indicates that there are micro-niches in the rock that are storing water for extended periods. Given the availability of water in the rock, it is possible for *T. erubescens* to access these moisture reserves (as outlined in Elliott *et al.* 2019).
- Details in Annual Research Report 1 (Elliott *et al.* 2018) and Part 2 of this report.

- **Conclusion:** Given the reduced conservative ecophysiological functioning during summer, when stomatal conductance thresholds are < 50 mmol.m^{-2}.s^{-1} (a threshold where stomata close on the leaf surface), it is likely that the species employs a drought avoidance strategy, which enables them to persist during summer periods when the rocky substrate is highly water deficient.

3.1.4 Develop understanding of the environmental factors that underpin variation in plant function

Research outcomes:

- By understanding the environmental factors underpinning variation in plant performance, thresholds limiting ecophysiological functioning can be identified. Spatiotemporal variation in plant performance was quantified across 13 seasons over three years, which provided the capacity to evaluate environmental factors underpinning environmental dynamics causing declines in plant performance and ultimately their survival.
- In summary, the results from Section 3.2.1 demonstrate increased plant function during winter compared to summer, and differences between north and south aspects.
- Stomatal conductance was related to rainfall (from climate data), soil moisture and temperature (from high resolution soil microclimate data), and leaf temperatures across the NE-facing and SW-facing aspect.
- Within a 4-week window prior to ecophysiological measurements, the number of rainfall events (>4 events) and average rainfall (>2.5mm) were significantly associated with increasing stomatal conductance responses in both aspects.
- Plants on SW-facing aspects are exposed to ca. 17% cooler (based on leaf temperatures) and ca. 95% wetter conditions, which are associated with increasing plant performance measures by at least 20-30%.
- Soil water potential and leaf temperatures are critical factors underpinning variation in plant function, with highest plant performance occurring in winter and the lowest performance associated with summer and autumn periods.
- Critical thresholds reducing stomatal conductance to <50 mmol m^{-2} s^{-1} were modelled at 35°C for leaf temperatures, and -32 MPa for soil water potentials – these thresholds were commonly exceeded in the NE-facing site.
- Details of research are in Part 2 of this report (Elliott *et al.* 2022).
- **Conclusions:** Soil water availability (measured by soil water potentials) and temperatures are key drivers for plant performance on the Koolyanobbing Ridge, with increased ecophysiological functioning measured on cooler/wetter SW-facing aspect. These locations may provide suitable baseline habitat characteristics that should be broadly taken into consideration for translocation planning.

3.1.5 Compare plant function (chlorophyll fluorometry, leaf gas exchange, and plant water status) of plants growing in natural and translocated sites.

Research outcomes:

- Comparisons against natural populations, help determine if translocated greenstock are demonstrating the capacity to establish, and the temporal requirement of establishing plant to become similar to naturally occurring plants. Additionally, these assessments may develop an understanding of the locations where older translocated plants are surviving and may provide a critical insight into the habitat requirements for future translocation planning.
- Ecophysiological assessments were conducted on cuttings that were planted in 2017-2020 translocation trials with the aim to compare plant function from establishing plants with plants in natural sites. Measurements were quantified using the same approach as outlined in Section 3.1.2, by quantifying stomatal conductance, leaf temperatures and chlorophyll fluorescence.
- A consistent trend was the decrease in ecophysiological functioning by at least 50%, as greenstock transition from winter into summer periods, with lowest ecophysiological functioning (< 50 $\text{mmol.m}^{-2}.\text{s}^{-1}$; Fv/Fm < 0.1; see Elliott *et al.* 2021, Figures 3.1.5a,b) observed after the first summer. As plants transition out of their first summer, ecophysiological functioning progressively became more similar to natural sites after two years.
- Details of research are in Annual Research Reports 2-4 (Elliott *et al.* 2019; 2020; 2021) and Part 2 of this report.
- **Conclusions:** Survival through the first summer is currently the largest bottleneck in newly planted greenstock. By measuring ecophysiological functioning, there is evidence that greenstock were capable of performing similarly to natural sites after two years. It is recommended that intervention measures should be undertaken prior to the onset of the first summer period to increase plant survival – as we have demonstrated that water stress and temperature decrease physiological functioning - management strategies should consider irrigating sites to increase water availability, and shading plants to decrease temperature of plants.

3.2 *Soil – nutrient acquisition interactions*

3.2.1 Assess the chemical and physical properties of soils from within natural *T. erubescens* populations.

Research outcomes:

- Defining the properties of soils will provide a greater understanding of the growth media that the species survives in and indicate what elements of the soils at translocation sites or in the nursery may need to be considered or altered to improve growth and survival.
- Soil chemical and physical composition analyses show a generally infertile and acidic soil of similar physical structure, but dissimilar chemical composition among locations.

- Ridge-top soils were generally associated with higher calcium (Ca) and magnesium (Mg) cations and nutrients.
- Soils sampled underneath and adjacent to *T. erubescens* plants were associated with higher mineral composition, specifically in relation to iron (Fe) and boron (B) concentrations.
- Investigations are based on low resolution of samples, and thus only present limited assessments of underlying soil chemical and physical composition.
- Details of research are in Annual Research Report 3 (Elliott *et al*. 2020).
- **Conclusion:** Higher Fe-concentrations in soils underneath *T. erubescens* may have important implications for their mineral nutrition. Further investigation into mineral uptake are detailed in Section 3.2.2.

3.2.2 Develop understanding of the importance of varying soil properties on plant survival and growth

Research outcomes:

- An understanding of the influence of soil properties on plant survival and growth provides information on what elements may be used, tolerated or required by plants and therefore indicate potential avenues for improving translocation site or nursery soils and nutrient supplements.
- Mineral composition in leaves in six co-occurring species (including *T. erubescens*) was determined from ridge top, BIF cliff/ rock outcrops, and adjacent slope ecotypes.
- There was clear separation of species sampled from the ridge top and slope locations, with the cliff locations classified as overlapping due to the plant species sharing similar leaf tissue composition traits with both the ridge top and slope locations. Leaves of *T. erubescens* and *B. arborea* had lower total N, P, K and Ca composition in comparison to the four other species, which is likely due to the species occurring on shallow soil substrates on BIF.
- Higher levels of Fe in *T. erubescens* compared to the other species confirms that the species are taking up the Fe from the soils.
- Investigations are based on low resolution of samples, and thus only present limited assessments of underlying plant leaf tissue composition.
- Details of research are in Annual Research Report 3 (Elliott *et al*. 2020).
- **Conclusions:** Given *T. erubescens* are absorbing Fe from the soil, this element was further utilised in soil treatments for nursery propagation of cuttings.

3.2.3 Provide data to support soil treatments aiming to improve the establishment and growth of plants in translocated sites.

Research outcomes:

- The capacity to supplement or ameliorate soil properties at the nursery phase or at translocation sites can be important in improving growth and survival if certain elements are required for optimal plant function. Based on the available information on soil chemical properties, the soils used for translocation were supplemented with an iron fertiliser.

- Soil treatments that used iron fertiliser as a supplement (2017 translocation: iron chelate supplement; 2018 translocation: Fetrilon Combi2) during the propagation phase, did not show improved greenstock performance under nursery conditions.
- Soil supplement treatments did not show improved greenstock establishment in translocation sites, due to poor survival rates observed the first summer after planting.
- Details of research are in Annual Research Reports 1 and 2 (Elliott *et al.* 2018; 2019).
- **Conclusion:** Soil supplement treatments did not appear to improve greenstock establishment under the field conditions tested, however, the benefit of soil treatments may need to be tested if the conditions of translocation were altered (e.g. irrigation).

3.3 *Soil biological function in natural and translocation sites.*

3.3.1 Assess biological communities of soils where *T. erubescens* grow

Research outcomes:

- The biological communities of soils consist of bacteria, fungi, viruses, archea and protists, which can influence plant survival and function if plant species have mutualistic-beneficial relationships with these microbes. These relationships may afford plants access to nutrients or drought and pathogen resistance. Understanding species diversity and composition of the soil microbe community can identify if this community is unique to the root zone area of *T. erubescens* and may suggest that there are specific relationships that need investigation, as this may indicate a potential path to improving translocation success.
- Genomic analysis of soil samples suggests that there are distinct bacterial and fungal communities under *T. erubescens* plants (162 bacterial and 187 fungal species) in comparison to locations that are not associated with *in situ* plants (189-236 bacterial and 98-233 fungal species), but all sample locations at Koolyanobbing Range were dominated by very low frequency species (<0.05% relative abundance).
- Details of research are in Part 2 of this report (Elliott *et al.* 2022).
- **Conclusion:** Whilst recognising distinct bacterial and fungal communities in the soils associated with *T. erubescens*, the functional significance of this result is not clear. For example, further research would be required to understand if *T. erubescens* is dependent on this soil microbial community (or certain species) for growth, and whether soils at potential translocation sites require this microbial community to support *T. erubescens*.

3.3.2 Assess the frequency and type of mycorrhizal associations of *T. erubescens*

Research outcomes:

- Mycorrhizal associations provide strategies for plants to obtain resources (e.g. nutrients, and water) from the soil. Understanding the presence of these associations in adult plants could be essential for determining inoculation regimes for optimising growth and establishment processes, particularly in newly planted greenstock in translocation sites.

- **Conclusion:** From rock excavations outlined in Section 3.1.3, roots (*n* = 9) were sampled from underneath *T. erubescens* plants (*n* = 2) and inspected for the presence of mycorrhizal associations using a high resolution compound microscope. The investigation did not detect any mycorrhizal associations on root surfaces.

3.3.3 Compare soil biological diversity and function between natural and translocated sites.

- Soils were only collected from natural sites (as described in Elliott *et al.* 2019) for the project, therefore a comparison with translocated sites could not be made. The lack of collection from translocated sites was due to the limited number of surviving greenstock of suitable age or similar histories at the end of the project, which restricted a robust sampling design. Additionally, the sampling process would have been destructive and would have prevented further long-term monitoring of the surviving greenstock. If future circumstances permit this type of sampling at translocation sites (e.g. robust sampling design without destruction), then the outcome would indicate whether *T. erubescens* is dependent on this soil microbial community (or certain species) to support growth.

3.3.4 Provide data to support soil inoculation aiming to improve the establishment and growth of plants in translocated sites.

Research outcomes:

- Many Australian plants have associations with microbial and fungal communities and understanding these interactions is important for the potential improvement of propagation and/or translocation success. Determining the magnitude of any effect will provide evidence of the importance of microbial and fungal relationships. As an initial step, the effect of inoculating nursery soils during propagation with soils from the natural population were tested.
- Soil inoculation during the propagation phase (1:8 field soil to potting mix), did not show improved greenstock performance under nursery conditions.
- Details of research are in Annual Research Report 1 (Elliott *et al.* 2018).
- **Conclusion:** Soil inoculation treatments (2017 translocation) did not show improved greenstock establishment in translocation sites, due to poor survival rates observed the first summer after planting. However, the benefit of soil inoculation may need to be tested if the conditions or information around translocation changed (e.g. irrigation or soil microbial communities).

Conclusion. Experimental translocation of *Tetratheca erubescens*

Key outcomes from these experimental translocations

Using an experimental framework in translocations provides an opportunity to test and compare techniques or protocols, and have been shown to underpin successful translocations (Silcock *et al.* 2019). Therefore, the response of the various factors being tested can be used to better formulate and improve best practice (Commander *et al.* 2018). The national guidelines for translocation of threatened plants in Australia outlines performance criteria that are desirable/appropriate to achieve for translocations (Commander *et al.* 2018). Translocation performance criteria can be based on several key ecological outcomes and can include:

- Persistence of sufficient individuals over specified timeframes.
- Multi-season flowering, fruiting and recruitment of subsequent generations.
- Maintenance of demographic processes.
- Persistence through fire/drought cycles.

The research outcomes of the experimental translocations of *T. erubescens* can be summarised – as a proxy – against some of these key performance criteria outlined under the national translocation guidelines (Commander *et al.* 2018) as follows:

- Persistence of individuals (up to 4 years) was achieved (87 plants). This research program led to the development of translocation protocols that support the establishment, survival, and persistence of individuals under natural environmental conditions. As these were experimental circumstances with the objective of "developing methods to support translocation", these data are not suited to evaluating whether a sufficient number was established. However, translocations were installed with a genetic profile that achieved an appropriate provenance sourcing approach that maximised the available genetic diversity (i.e. in any year, 32-68% of greenstock planted were unique genotypes; 93% of surviving greenstock were unique genotypes).
- Multi-season flowering and fruiting of individuals were achieved. The experimental translocation demonstrated that reproductive capacity can be achieved in a shorter timeframe (4-16 months after planting) than that observed from the natural population (seedlings are >3 years old). Recruitment of subsequent generations is a medium-term criterion (4-10 years) and has not yet been detected in the short timeframe of this experimental translocation. As such, recruitment should not be expected at these early stages, despite the initiation of reproduction, because there has been limited quantification of seed production; evidence of incorporation of seed into the soil seedbank; or realisation of an event that could initiate recruitment.
- The maintenance of demographic processes is a long-term performance criterion (>10 years) related to the establishment of a self-sustainable population, and despite the short timeframe there were observations of successful pollination (i.e. seed production) and recruitment from directly sowed seeds stimulated by natural rainfall in the translocations. Here, we have been able to demonstrate evidence of certain demographic processes that are functioning, through the monitoring of key traits in translocations, during early establishment phases.

- The persistence through fire/drought cycles is also a long-term performance criterion (>10 years). There was an indication of individual survival during periods of below average rainfall, thus demonstrating a capacity for translocated plants to persist under seasonal "drought conditions" despite the short timeframe so far. Further work will be required to extract disturbance (i.e. fire) or climatic (i.e. multi-year drought) responses to long-term demographic cycles, through long-term monitoring or experimental research.

Context of these experimental translocations

Comparisons of translocation research among species are difficult to make due to the diversity of species, habitats, propagules and installation and management approaches that make evaluations a challenge (Silcock *et al.* 2019). From a national perspective, these experimental translocations (87 plants; 1.5 – 4.5 years old) appear to sit within the top 45% of Australia's translocation outcomes, in terms of having >50 plants surviving more than one year (Silcock *et al.* 2019). However, the short-term success of the experimental translocations was in the lower 28% nationally (<50% survival after one year) due to low plant survival after the first summer. A generalised comparison among threatened species that occur in the same habitat (banded iron formations; see Appendix 3) can be made, however, we can only provide a brief context for the translocation research program on *Tetratheca erubescens* because translocations of banded ironstone species are limited and vary widely based on:

- species (genera and lifeform)
- translocation start year and location (climate conditions)
- translocation habitat (disturbed or natural; suitable or unsuitable)
- scope of experimental research program (limited vs extensive)
- length of monitoring period (one vs many years)
- irrigation use (yes or no)

With these differences in mind, we can better attempt to understand the context of translocation efforts observed in *T. erubescens*, in broad terms, even over the relatively short-term of this experimental research program.

- Seedling emergence (2.5%) was higher than a sister species, in the same habitat at another location (*T. paynterae* subsp. *paynterae*; 2%) and was lower than that of a shrub (*Ricinocarpos brevis*; 3.6%) that had the advantage of irrigation and a more homogenous habitat (waste rock landform) for its translocations (see Appendix 3).
- Seedling survival, however, was lower than that of the sister species, and much lower than that of the shrub species that was irrigated (see Appendix 3).
- The number of tubestock survivors (<3%) was within the range of survival for the other species that were also non-irrigated (0-96%), however the broad range in survival rates indicates the complexity of conducting translocations on banded ironstone formations (see Appendix 3) for the reasons summarised above. Consistent factors in translocation success and plant survival for banded ironstone species relate to moisture availability, habitat suitability, climatic conditions at time of planting and during the establishment phase, all of which were also applicable to *T. erubescens*.

PART 2: RESEARCH RESULTS 2021-2022

Program 2. Translocation and monitoring

2.1 *Optimising translocation approaches*

Summary of translocation logistics – 2017-2020

The landform habitat characteristics of the 2017-2020 translocation sites are summarised in Table 2.1a. In late 2017, the immediate area of T19 became unstable and the presence of mining activities close to and above the T19 area presented unsafe conditions for personnel to conduct ongoing monitoring (monitoring February 2018 incomplete). An additional translocation site (T24) was approved for use as a translocation site in 2018-2020 (Table 2.1a). The overall numbers of seed and greenstock trialled at each site for each translocation year are summarised in Table 2.1b. An activities schedule for each translocation is presented in Table 2.1c and outlines the timing of installation and monitoring of the translocations. The type of experimental treatment tested for the direct seeding or planting of greenstock in each year of translocation are in Table 2.1d. Protocols or work instructions for the planning, implementation and monitoring of experimental translocations for *T. erubescens* are in Figures 2.1a-2.1d.

Comprehensive details of the 2017-2020 translocation trials are in Annual Research Reports 1-4 (2018-2021) or below in Part 2 of this Final Report. The national guidelines for translocation of threatened plants in Australia details a comprehensive step-by-step guide to plant translocations that will provide further information to compliment the content of this report (Commander *et al.* 2018).

Seed collections:

Seed collections were critical for completing experiments across all three research programs, particularly for translocation-based activities. Several competing factors needed to be considered when planning and managing project logistics, and prioritisation of activities was often necessary due to the availability of seed from this threatened species. There was:

- annual variation in the number of seed available and their quality from which to collect in the natural population (see Annual Reports 1-4; Elliott *et al.* 2018-2021).
- strict licensing requirements and conditions for seed collection (e.g. restricted collection amounts; 20% of collection to be submitted to DBCA Threatened Flora Seed Centre).
- specific guidelines on propagule sourcing (i.e. genetic provenance) for each translocation site (i.e. conditions of approved translocation proposal).

Table 2.1a. Summary of specific landform habitat details for each translocation site.

Site	Latitude	Longitude	Geology	Translocation potential (no. plants)	Distance to extant plants	Model strength (BGPA 2015)*
T6	-30.87245	119.60269	Canga/weathered haematite	<300	<0.1km	<0.3
T18	-30.88656	119.61919	BIF (high iron)	<200	0.7km	0.45-0.5
T19	-30.87145	119.60642	-	50	<0.1km	<0.3
T21	-30.87394	119.60513	BIF (20% iron)	75	<0.1km	0.55-0.6
T23	-30.87150	119.60637	BIF (20% iron)	150	<0.1km	<0.3
T24	-30.87417	119.61111	Canga	150	0.18km	0.3-0.5

* the higher the number the higher the predicted likelihood of habitat matching by the model for *Tetratheca erubescens* (Miller 2015)

Table 2.1b. Summary of the number of seed sown and greenstock planted for each of the six translocation sites, in each of the four years of translocation.

Year	Propagule	T6	T18	T19	T21	T23	T24	TOTAL
2017	Seed sown	1120	570	160	180	230	-	2260
	Greenstock	273	312	49	-	137	-	771
2018	Seed sown	320	320	-	200	-	-	840
	Greenstock	293	341	-	106	120	218	1078
2019	Seed sown	400	800	-	400	400	-	2000
	Greenstock	99	255	-	57	47	42	500
2020	Seed sown	300	300	-	-	-	300	900
	Greenstock	162	295	-	88	50	128	723

Table 2.1c. Summary of installation (black) and monitoring (grey) periods for each year of the project. Four separate translocations were installed (2017-2020) at 5-6 sites (T6, T18, T19 (only 2017), T21, T23, T24 (not 2017)) and each site was monitored post-installation (8 weeks), each winter (Aug), each spring (Oct-Nov) and each summer (Feb).

	J	F	M	A	M	J	J	A	S	O	N	D
2017								■		▒		
2018		▒				■		▒		▒		
2019		▒				■		▒			▒	
2020		▒			▒	■		▒		▒		
2021		▒				▒				▒		

Table 2.1d. Summary of the type of experimental treatments implemented in each year of translocation for seed and greenstock.

Year	Propagule	Laboratory or Nursery			Field-based
2017	Seed	Warm stratification	Soil seedbank vs fresh seed	-	Irrigation applied through hand watering
2018		Warm stratification	-	-	Drill holes vs cracks
2019		Warm stratification	-	-	Drill holes vs cracks
2020		Warm stratification	-	-	Drill holes vs cracks
2017	Greenstock	Iron supplement	Soil inoculum	Pot type	Irrigation applied through hand watering
2018		Iron supplement	-	-	Shade
2019		Cutting vs seedling	-	-	Water crystals
2020		Cutting vs seedling	-	-	-

Propagation preparation:

1. Source propagation material from natural populations (seed collection or cuttings during plant growing period) according to collection license conditions, which maximises the genetic diversity of the collection.
2. Maintain a database of source material location information for each propagule produced to ensure the correct source (i.e. provenance) is delivered to the approved translocation site (i.e. determined in the approved translocation proposal).
3. Greenstock grown in accredited nursery
    a. 1:1:2 mix of peat, fine perlite and fine river sand used to initiate propagation (cell trays), on heated beds (25°C) for root development and have a misting or wicking delivery of water.
    b. 5:2:1 mix of native potting soil, fine river sand, fine perlite used to pot on greenstock for translocation (Biodegradable pots: Fertil™ 50mm x 90mm).
    c. 8:2:1 mix of native potting soil, coarse river sand, coarse perlite used to pot on greenstock for permanent collections in the nursery (plastic pots).
    d. Regular watering is required but do not overwater (sensitive to root rot and soil pathogens that like hot/wet soils), plants in pots should partly dry rather than be constantly wet. Regulate watering to suit temperature changes in winter and summer.
    e. No adverse effect to fertilising (native plant fertilisers) or pest control (oil).

4. Drill appropriately sized holes into the rock:
    a. Biodegradable pot size is 50mm diameter and 90mm height.
    b. Drilled hole larger than the pot (60-70mm diameter and 100-120mm depth).
    c. Drill holes at an angle to encourage water collection on the pot surface and funnelling of water to the roots (i.e. >30 degree angle; Figure 2.1a).

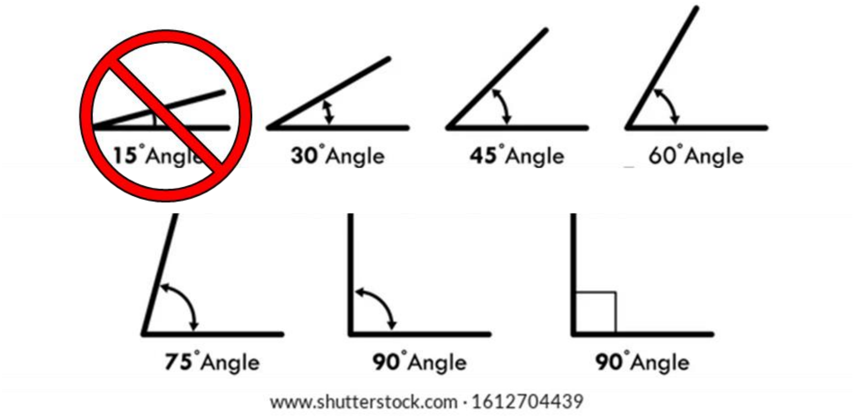


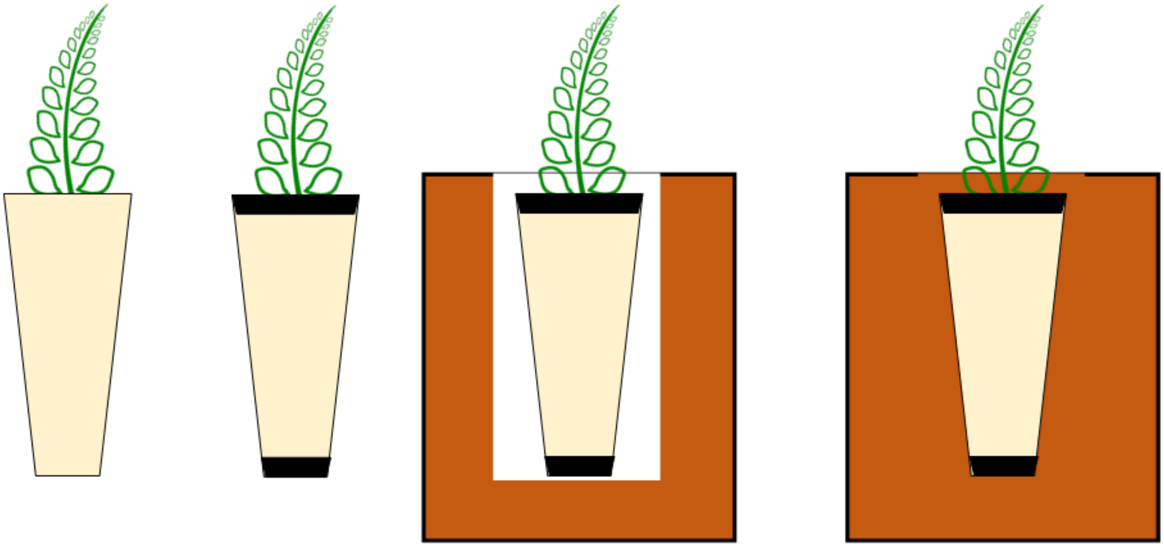
Figure 2.1a. Angles to drill holes into the rock to accommodate greenstock pots.

5. Placement of the drill holes on rock strata should consider the research outcomes of Section 2.1.3 (Annual Report 2-3; Part 1 & 2 of this report), which highlights the local habitat characteristics that were more likely to support tubestock survival.
6. Acclimatise greenstock to site conditions 1-6 weeks ahead of planting
    a. Maintain regular watering (do not stress plants).
    b. Protect from frost and herbivory.
    c. Plants to be well watered 2-3 days before planting.
    d. Plants to be checked for pests (e.g. scale).
    e. Pots to be checked for weeds.
7. Cliff/rock planting requires topsoil (not base rock or waste rock soil) for filling that is:
    a. weed free.
    b. sieved to remove rocks, small stones and twigs.
    c. dry (e.g. in oven <50°C) to allow pouring of topsoil into narrow cavity around tubestock that prevents formation of any air gaps and root exposure later.

Instructions for planting:

1. Ideally, holes should already be moist, so fill with water 1-2 days before planting, especially if it has not rained prior to planting.
2. Planting biodegradable pots (Figure 2.1b):
    a. Remove the bottom of the pot (allows roots access to fissures).
    b. Remove the top 0.5-1cm of pot, so the surface soil sits above the pot margin (this prevents wicking away of moisture from pot after planting).
    c. Gently place plant and pot into the hole.
    d. Pack topsoil around the sides of the pot so there are no air gaps.
    e. Pack topsoil on top of the pot so no potting mix (or pot) is visible.
3. Gently **water** the day of planting: slow spray to allow absorption and prevent water running off (>50mL potable water to be delivered at planting).

4. Glue individual pot tag to the LEFT of the plant.
5. Map plant location on translocation site map.
6. Record plant survival and growth (regularly).



Remove bottom
and top of bio pot

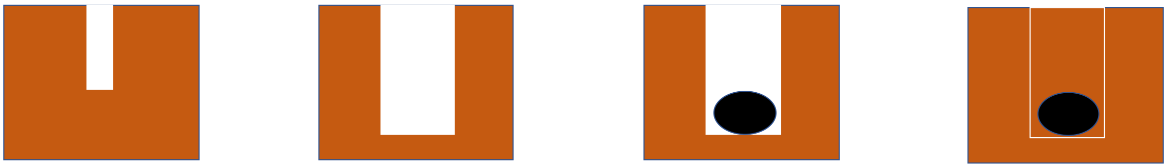
Place in drilled hole

Completely pack with soil
- Sides and cover top

Figure 2.1b. Instructions for planting greenstock in biodegradable pots.

Instructions for seed sowing:

1. Cracks or fissures need to be deep enough to contain seeds and the soil on top
2. Gently place seeds into large enough natural crack, spread along the crack.
    a. If not large enough, use a hammer and chisel to widen and deepen the crack for seeds to be placed inside **[depth ~ 2x the size of seed]**.
3. Cover seeds with topsoil to prevent them from being removed by ants or washed away (Figure 2.1c).
4. Glue marking tag **below crack** to the LEFT of the first seed.
5. Spray paint a mark to the RIGHT of the last seed.
6. Map seed location on translocation site map.
7. Gently water with misting spray the seed locations (secures soil in place).
8. Record seedling emergence regularly, particularly after rains (August-October).
9. Record seedling survival and growth (regularly).



Widen and deepen natural fissures
- Depth ~ 2x seed size

Place seed in fissure

Cover seed with soil

Figure 2.1c. Instructions for sowing seeds into cracks or fissures.

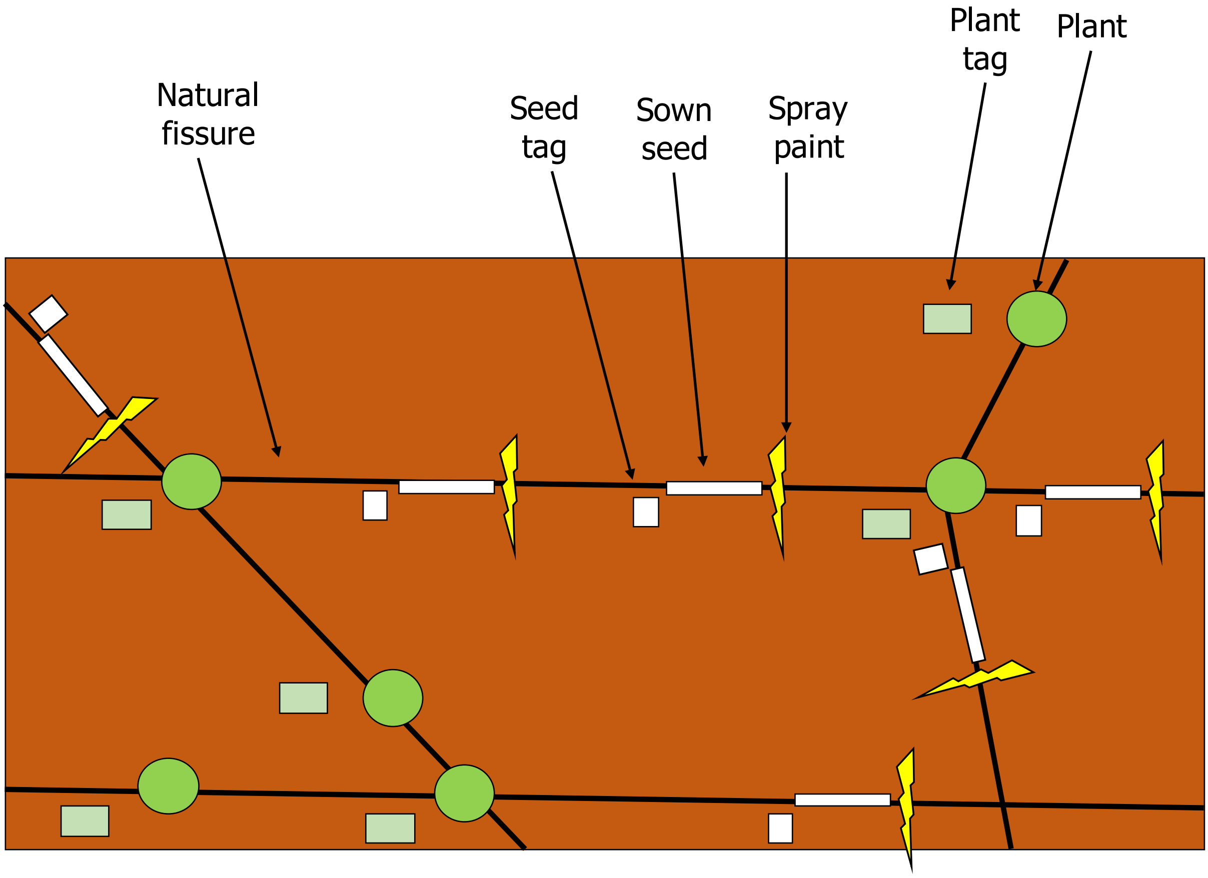


Figure 2.1d. Example of a mapping scenario to be documented so that monitoring data can be accurately recorded and tracked for each individual.

2.1.1 Assess the effectiveness of treated *in situ* sown seeds for undertaking translocations.

Research outcomes:

- Seeds are a critical plant source for revegetation programs. As we have determined, an effective pre-treatment to alleviate dormancy through stratification, and further enhance germination using KAR, trials were conducted to determine the capacity of seeds to recruit under field conditions following pre-treatment. We assessed the effectiveness of treated seeds through burial trials (in T6 and T18) and in sowing lines along cracks or artificial drill holes across translocation sites (see Part 2: Table 2.1b).
- Seed burial trials provide a means to quantify the persistence of seeds under field conditions. Seed persistence is critical for understanding population sustainability, as this process determines the ability of a species to survive in the soil seedbank, particularly after death of the parent plant.
- Burial trials demonstrate seeds transition from a dormant to a non-dormant state within a year of burial. From untreated seeds, 80% of dormant seeds transition to non-dormancy, whilst from treated/stratified seeds, 100% of dormant seeds were non-dormant after 485 days of burial.
- Irrigation supported a germination pulse shortly after sowing seeds, with 8% of seeds germinating after 70 days (compared to 0% in non-irrigated burial locations).

- Seeds can persist for at least three years in the soil seedbank, with modelled p50 (viability declines of 50%) of at least 1170 days.
- Seed germination pulses appear to occur during winter seasons – there are benefits to stratifying seeds, due to the fast germination pulse after sowing (12.5% after 56 days; see Field burial trial 1).
- Despite the observed germination responses, the bottleneck appears to be the transition from the germinated seed stage to the emerged and surviving seedling stage.
- *In situ* sown seeds resulted in seedling emergence (overall 2.5%) and survival (overall <1%) at most translocation sites (except T23).
- Survival of seedlings was poorest after their 1st summer (<4%)
- Translocations can be undertaken with pre-treated seeds sown *in situ* into drill holes or large cracks because seedlings will emerge under the appropriate environmental conditions, and seeds will persist in the environment for multiple years, ensuring opportunities to recruit in the future.
- Patterns in persistence and recruitment were assessed in seven co-occurring species. Experiments were conducted on rocky substate (the preferred niche of *T. erubescens*) and adjacent located slope niches (see Field burial trial 2). In total, five plots were installed per species in both ecotypes, with seed persistence, germination, emergence and survival monitored after 72 and 112 days.
- There was variability in the preference for niches, with species such as *Acacia tetragonophylla*, *Allocasuarina campestris*, *Banksia arborea*, and *Senna artemisioides* subsp. *filifolia* demonstrating the capacity to germinate and emerge in the slope niche, and species such as *Austrostipa nitida*, *Grevillea hookeriana* and *T. erubescens* generally demonstrating a preference for germination in the rock niche (germination 0-75%; 0% emergence).
- The research demonstrated that *T. erubescens* has a conservative recruitment strategy, whereby there is a trade-off between low germination (<15%) and high seed persistence (>90% after 112 days), in comparison to the other species that demonstrated high germination (up to 80%), but low persistence (e.g. *A. tetragonophylla*, *S. artemisioides* subsp. *filifolia*, *and Solanum lasiophyllum* (<20%). Despite the high germination from co-occurring species, there was low emergence (<40%) and high mortality in seedlings (100% mortality after 112 days). These responses could lead to high seed depletion rates in the seedbank, and the requirement for high annual seed production in these species to maintain their populations.
- The low germination and high persistence responses in *T. erubescens*, reflects a favourable strategy for plant recruitment in a water-limited ecosystem, as seed losses are minimised over time.
- The outcomes here highlight species differences, which were poorer seed persistence and higher mortality rates of seedlings after emergence, in co-occurring species when compared to *T. erubescens*.

Direct seed sowing at translocation sites

Sowing seeds into natural habitat (rock cracks or holes) or modified natural habitat (drill holes) at translocation sites resulted in seedling emergence that varied from year to year. Key outcomes for translocations were:

- Method – evaluation of seed/soil washing out of cracks after rain was important in choosing safer locations (deeper cracks or drill holes) in the following years (see Elliott *et al*. 2020).
- Emergence – seedlings were observed at all translocation sites over the five years, indicating their suitability for the recruitment phase. Of the 1000's of seed sown *in situ* (Part 2: Table 2.1b), emergence ranged from 17-80 seedlings per year.
- Mortality of seedlings was highest after their first summer (>80% mortality).
- Seedlings can survive multiple summers (reached 3-4 years of age).
- Survival of seedlings was greatest after their 2nd or 3rd summers (i.e. 48-93% survival).

Field burial trial 1 – Seed persistence of **Tetratheca erubescens**

Background and method

Seed burials provide a means to quantify the persistence of seeds under field conditions. This knowledge is critical to help quantify seed turnover rates through dormancy cycling, germination or decline of viability in the field after natural dehiscence/ dispersal, or when sown artificially for management programs (e.g. translocation, and/or restoration). Understanding seed persistence is critical for understanding population sustainability, as this process determines the ability of a species to survive in the soil seedbank, particularly after death of the parent plant and thus safeguarding population sustainability and genetic diversity over time.

To quantify seed persistence, nylon mesh bags containing seeds were buried under 5-10 mm of topsoil at two translocation sites in 2019 (T18; during early Winter, 13-23rd June) and 2017 (T6; during late Winter, 9-16th August). The first extraction was approximately 72 days and 56 days after sowing for T18 and T6, respectively.

In T18, the effects of alleviating seed dormancy were tested via seed burials. While alleviated dormancy was predicted to increase seed germination rates in the field, untreated/dormant seeds could demonstrate delayed germination over time due to seed dormancy. In total 16 burials per treatment were installed into pre-drilled holes in the rock strata. All seeds in T18 were exposed to natural rainfall.

In T6, the effects of irrigation were tested via seed burials, whereby burial locations were irrigated with approximately 10-30ml of water weekly for 12 months. It was expected that increased moisture availability, would result in higher germination rates compared to the non-irrigated treatments. It was also expected that seeds could be less persistent through irrigation, as continuous moisture application could lead to seed deterioration if moist seeds remain ungerminated. In total 32 burials (each bag containing 10 seeds) were installed throughout the rock strata, whereby half of the burials were non-irrigated. All seeds had undergone stratification to alleviate seed dormancy, prior to field installation.

Seeds had undergone viability testing, and fill-rate assessments prior to sowing in the field. Four replicate bags of each treatment were extracted ca. 56 days and then yearly for up to three years during the Spring period. After burials were extracted from the field, seeds were carefully removed from bags and inspected for germination under a dissection microscope Ungerminated seeds were then surface sterilized using a 2% w/v calcium hypochlorite ($Ca[OCl]_2$) solution for 30 minutes, alternating for 10 minute cycles under vacuum (e.g. on/off/on at -70 kPa), and then rinsed in sterile deionized water prior to being incubated at 20°C for 30 days. Seeds that germinated during the incubation period were classified as alive and non-dormant. Seeds that did not germinate after the 30 day period were then cut-tested in order to determine if ungerminated seed were either alive and dormant, or dead. Viable seeds were classified as the sum of alive and, non-dormant, and dormant seeds that remained ungerminated following burial.

Results

Site T18

__Seed performance prior to burial__: Prior to sowing in the field, untreated/control seeds germinated up to 54%, with 46% being dormant. Stratified seeds were germinating up to 72%, with 26% for dormant seeds and 2% dead.

__Seed fates during burial:__ Untreated seeds germinated to 2.5% after 56 days. After 485 days, germination from burials was 17.5% from bags. The increase in germination was explained by an increase in alive, non-dormant seed of 67.5% compared to 54% when seeds were sown. There was an initial pulse of germination after 56 days for stratified seeds, with 12.5% of seeds germinating after the first winter. After 485 days, germination of stratified seeds was 22.5%. Dormancy declined from 19% to 0% after 485 days of burial, with the amount of non-dormant seed increasing to 80% after 485 days of burial. While germination was low, there was a slight benefit to stratifying seeds, due to the initial germination pulse that occurred after 56 days, in contrast to untreated seeds. Untreated seeds however, transition out of the dormant seed state after a year of burial, as they transition through spring, summer and autumn seasons.

Site T6

__Seed fates at start__: Prior to sowing in the field, *ex situ* tests of stratified seeds showed germination up to 61%, with 36% being dormant and 3% were dead.

__Seed performance prior to burial:__ After sowing in the field, germination was higher for irrigated seeds at 72 days (8%) compared to non-irrigated seeds (2.5%). Seeds buried for between 72 and 816 days had similar germination between irrigated and non-irrigated treatments (less than 10%, Figure 2.1.1a), with natural rainfall likely contributing towards increases in germination from non-irrigated burial locations. After 1170 days of burial, locations that were irrigated previously demonstrated highest germination of 30% compared to 10% in non-irrigated locations. For both irrigated and non-irrigated burial locations, there were consistent declines in dormancy and increases in the number of dead seeds (Figure 2.1.1a). Overall, it is possible that the low germination in irrigated locations was due to the low volumes of water applied to seeds (see Elliott *et al.* 2018).

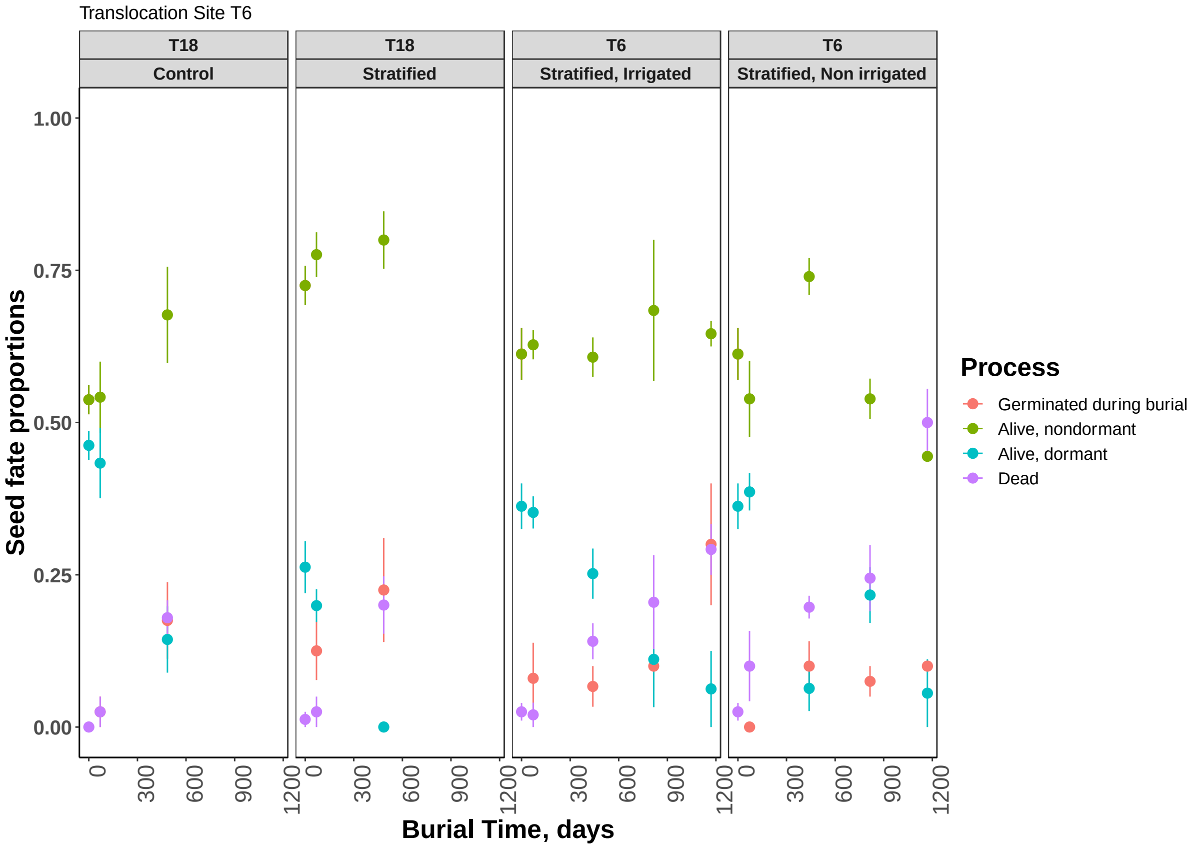


Figure 2.1.1a. Seed fate – seeds that germinated during burial, remained alive and non-dormant or dormant, or were dead over a three year burial period in two translocation sites. In T18, comparisons were drawn between seeds in dormant and non-dormant states, as determined by the pre-treatment prior to sowing in the field: untreated/control and stratified seeds. In T6, all seeds were stratified to alleviate seed dormancy prior to being irrigated or not. All point estimates represent means with standard error bars, *n* = 4 burials (*n* = 10 seeds/burial).

Seed persistence

When modelling persistence of seeds (see Figure 2.1.1b), all trialled treatments demonstrated declines in seed viability after the first year of burial (ca. 20-25%), with no differences between untreated and stratified seeds in T18 ($F_{2,22} = 0.186$, $P = 0.831$), and marginally non-significant differences between irrigated and non-irrigated burial locations in T6 ($F_{2,34} = 3.218$, $P = 0.0532$). The predicted p50 values (parameters predicting 50% decline) were 1268 ± 214 days and 1193 ± 191 days for untreated/control and stratified seeds in T18, respectively; and 1522 ± 156 days and 2046 ± 257 days for non-irrigated and irrigated burial locations in T6, respectively.

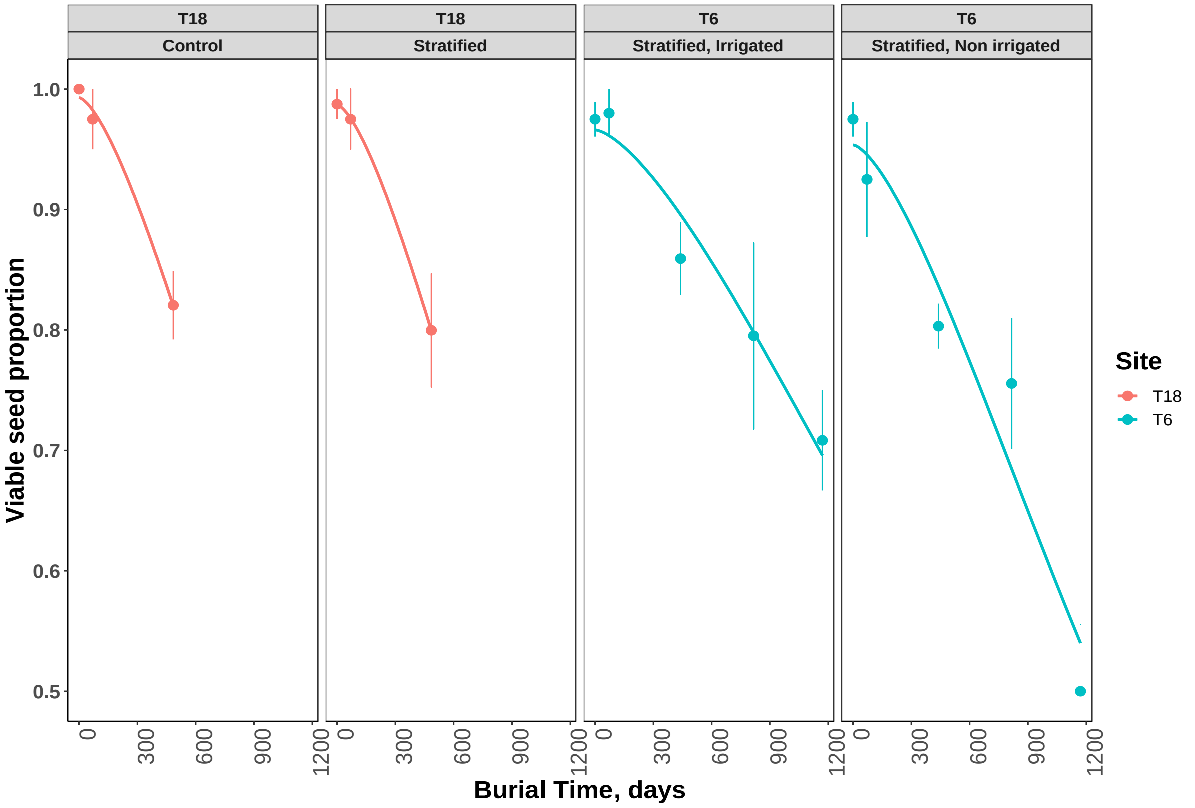


Figure 2.1.1b. Seed persistence as demonstrated by declines in viability during burial. In T18, comparisons were drawn between seeds in dormant and non-dormant states, as determined by the pre-treatment prior to sowing in the field: untreated/control and stratified seeds. In T6, all seeds were stratified to alleviate seed dormancy prior to being irrigated or not. All point estimates represent means with standard error bars, *n* = 4 burials (*n* = 10 seeds/burial).

Conclusions

Seed persistence analysis demonstrate the capacity of seeds to survive for 1193 days prior to a 50% loss of viability. Within this timeframe, seeds cycle out of dormancy within the first year and demonstrate the capacity to germinate in the soil seedbank, annually. We did not measure whether seeds cycle into dormancy (non-dormant seeds becoming dormant) – this would require seasonal sampling of burial bags. The largest recruitment bottleneck following germination was emergence, with field emergence at translocation sites lower than measured in burial bags (see Annual Reports 1-4; Elliott *et al.* 2018-2021). Low emergence rates have been previously reported in a number of arid zone species (e.g. Erickson *et al.* 2017; James *et al.* 2019; Lewandrowski *et al.* 2021), including BIF species (Commander *et al.* 2019; Merino-Martín *et al.* 2017). The successful transition from germination to emergence is dependent on the microclimatic niche into which seeds are either sown, or dispersed, with low rainfall and increases in temperature significant drivers for seedling losses (James *et al.* 2013). While the advantage for sowing seeds is the ability for seeds to persist in the seedbank across a number of seasons, this effort needs to be weighed critically against the capacity of the seeds to germinate and emerge and the reliance on suitable rainfall and temperature conditions for successful establishment.

Field burial trial 2 – Comparison of species on rock and adjacent slope microsites

Background and methods

The low germination in *T. erubescens* observed through the burial trial (and the low emergence responses observed in translocation sites) raised questions on whether co-occurring species would display similar patterns for seed persistence and recruitment in the landscape. As such, we aimed to determine patterns of seed persistence in two habitat types, a rock niche (preferred habitat of *Tetratheca erubescens*) and an adjacent slope. We used eight co-occurring species (*Acacia tetragonophylla – ACATET*, *Allocasuarina campestris – ALLCAM*, *Austrostipa nitida – AUSNIT*, *Banksia arborea – BANARB*, *Grevillea hookeriana – GREHOO*, *Senna artemisioides* subsp. *filifolia – SENART*, *Solanum lasiophyllum – SOLLAS*, and *Tetratheca erubescens – TETERU)* for comparisons*.* To determine patterns of persistence, we monitored seedling recruitment and seed viability for seeds buried in a single translocation site (T18). Seedling recruitment was determined by quantifying germination during seed burials, and counting total emergence and survival from the emerged seedlings. Viability was determined by extracting seed burials from the field, and assessing ungerminated seeds in the laboratory through germination trials and cut-testing.

For each species, five replicate persistence lines were installed in either rock (Figure 2.1.1c) or adjacent slope (Figure 2.1.1d) ecotypes in winter (20th June, 2019). In the rock ecotype, seeds were sown along cracks on rock benches approximately 10-20 cm in width and 20-30 cm in length, and covered with 10 mm of local topsoil. All persistence lines in the rock niche were clearly marked with a tag and a spray paint line indicating the start and end of the line (Figure 2.1.1c). In the adjacent slope ecotype, seeds were sown into 10-20 cm wide and 30-50 cm long hand-excavated furrows and covered with topsoil (Figure 2.1.1d). Each line was clearly marked with tag and a high-visibility string over the furrow. Two bags containing seeds were buried at the start of each persistence line. There were two monitoring intervals, 72 and 112 days, where the total number of emerged (living and dead) seedlings were counted and marked with toothpicks. The surviving seedlings were counted as the living proportion at each monitoring interval. Burial bags containing seeds were extracted and inspected, whereby the number of germinated seeds were counted, and ungerminated seeds assessed for viability in germination trials. Viable seeds would either germinate under optimal conditions following incubation in the laboratory, or present healthy (firm, white embryo and endosperm) after cut-testing.

All species were treated to alleviate seed dormancy prior to sowing in the field. This included immersing in hot water for 90 seconds (*ACATET* and *SENART*), after-ripening for 4 weeks and treating with a 24 hour seed soak in karrikinolide (*AUSNIT*, *GREHOO* and *SOLLAS*) or left untreated (*ALLCAM* and *BANARB*). Seeds of *Tetratheca erubescens* were sown as untreated (*TETERU-C*) or stratified to alleviate dormancy (*TETERU-S*). The total number of seeds per persistence line and burial bag was as follows: $n_{(ALLCAM)}$ = 30; $n_{(ACATET,\ AUSNIT,\ BANARB,\ SENART,\ SOLLAS)}$ = 20; $n_{(GREHOO)}$ = 15; and $n_{(TETERU\text{-}C,\ TETERU\text{-}S)}$ = 10.

Figure 2.1.1c. Persistence lines in the rock niche ecotype, with tag and spray paint markers indicating start and finish of each line. Each persistence line in the rock was approximately 10-20 cm in width and 20-30 cm in length.

Figure 2.1.1d. Persistence lines in the slope niche ecotype, with tag and high visibility string indicating start and finish of each line. Each persistence line in the slope ecotype was approximately 10-20 cm in width and 20-30 cm in length.

Results

Germination

There were general increases in germination over time with maximum germination occurring after 112 days in most species, and a preference for either slope or rock niches (Figure 2.1.1e). For species such as *A. tetragonophylla*, *A. campestris*, *B. arborea*, *S. artemisioides* subsp. *filifolia* and *S. lasiophyllum*, germination responses were higher (>40%) in the slope when compared to the rock niche (Figure 2.1.1e). Species, such as *A. nitida*, *G. hookeriana* and *T. erubescens* generally demonstrated a preference for the rock niche, however presented germination of 17%, 50% and 13%, respectively (Figure 2.1.1e).

Emergence and survival

Emergence from sown seeds was only observed in *A. tetragonophylla*, *A. campestris*, *A. nitida* and *B. arborea* in the slope niche after 72days (Figure 2.1.1e). Emergence was highest from *A. campestris* (35%), and never exceeded 2.5% in the other species. Of the 35% of seedlings that emerged in *A. campestris*, only 75% were surviving after 72 days, while of the 2.5% that emerged in the other species, 100% were still alive (Figure 2.1.1e). There was no additional emergence after 112 days in any of the species, and all seedlings that were surviving after 72 days and were dead after 112 days (Figure 2.1.1e).

Seed persistence

Seed persistence varied across species and the niches. Highest persistence on average of all species were presented in *T. erubescens*, with both untreated and stratified seeds presenting >90% after 112days of burial (Figure 2.1.1f). Species that were showing similar responses were *B. arborea* and *G. hookeriana* – although seeds in these species were generally presenting a higher persistence in the rock niche in comparison to the slope niche (Figure 2.1.1f). Species that demonstrated higher persistence in the slope were *A. campestris* and *S. lasiophyllum*, with seed viability declining to 72% and 53%, respectively, whilst in the slope, viability declining to 42% and 0% after 112 days. *Acacia tetragonophylla*, *A. nitida* and *S. artemisioides* subsp. *filifolia* were generally associated lower decline in seed persistence in the rock niche, compared to the slope (Figure 2.1.1f). Out of all species, *A. tetragonophylla* and *S. artemisioides* subsp. *filifolia* demonstrated the highest declines in viability in both niches.

Conclusions

For all species, recruitment was very low. While some species demonstrated the capacity to germinate as seasonal temperatures transition from winter cool to early summer heat, species were overall demonstrating recruitment failure, with no seedling survival after 112 days. The results demonstrate that species such as *A. tetragonophylla*, *A. campestris*, *B. arborea*, *S. artemisioides* subsp. *filifolia* and *S. lasiophyllum* displayed germination pulses following winter and spring rainfall, which could be explained by seeds having been pre-treated (except for *B. arborea*) to alleviate seed dormancy prior to sowing. For species that germinate, but fail to either emerge or to survive after emergence, could lead to decreases in seeds being available in the seedbank, and a necessity for yearly seed production and input into the soil seedbank.

In contrast, *T. erubescens* presented the lowest germination responses out of all species, which could be interpreted as a conservative recruitment strategy, even when seed dormancy was alleviated. The low germination could be explained by high diurnal temperature fluctuations on the ground and rock that are signalling a risky niche for recruitment (see Elliott *et al.* 2021).

Out of all species, seed persistence in *T. erubescens* on average was the highest after 112 days, indicating the capacity for seeds to be retained in the seedbank with a very low proportion of the seed cohort responding to winter and spring rainfall. This conservative recruitment strategy could be viewed as favourable in a water-limited ecosystem. If recruitment processes are to be optimised in the field, suitable niches in the rock would need to be identified in order to maximise germination and emergence processes. As seed germination is optimal at cool temperatures (15-20°C), but seeds need a moisture window of 14-21 days to support germination (Section 1.1.2 in Elliott *et al.* 2020), intervention strategies would need to investigate methodologies to manipulate the thermal and hydrological environment surrounding seeds.

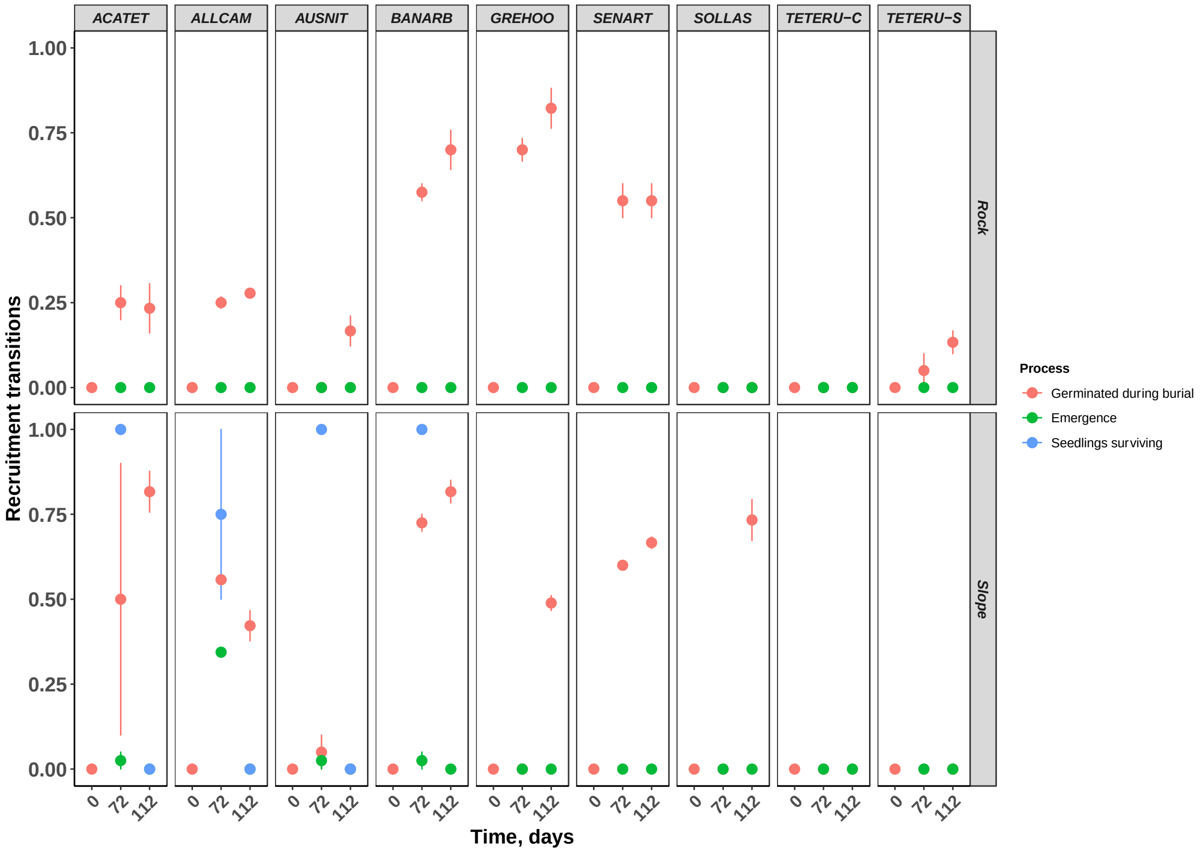


Figure 2.1.1e. Recruitment transitions (germination during burial, emergence and seedlings surviving) after 112 days in rock and adjacent slope niche ecotypes in eight co-occurring species (*Acacia tetragonophylla – ACATET*, *Allocasuarina campestris – ALLCAM*, *Austrostipa nitida – AUSNIT*, *Banksia arborea – BANARB*, *Grevillea hookeriana – GREHOO*, *Senna artemisioides* subsp. *filifolia – SENART*, *Solanum lasiophyllum – SOLLAS*, and *Tetratheca erubescens – TETERU-C*; *TETERU-S*).

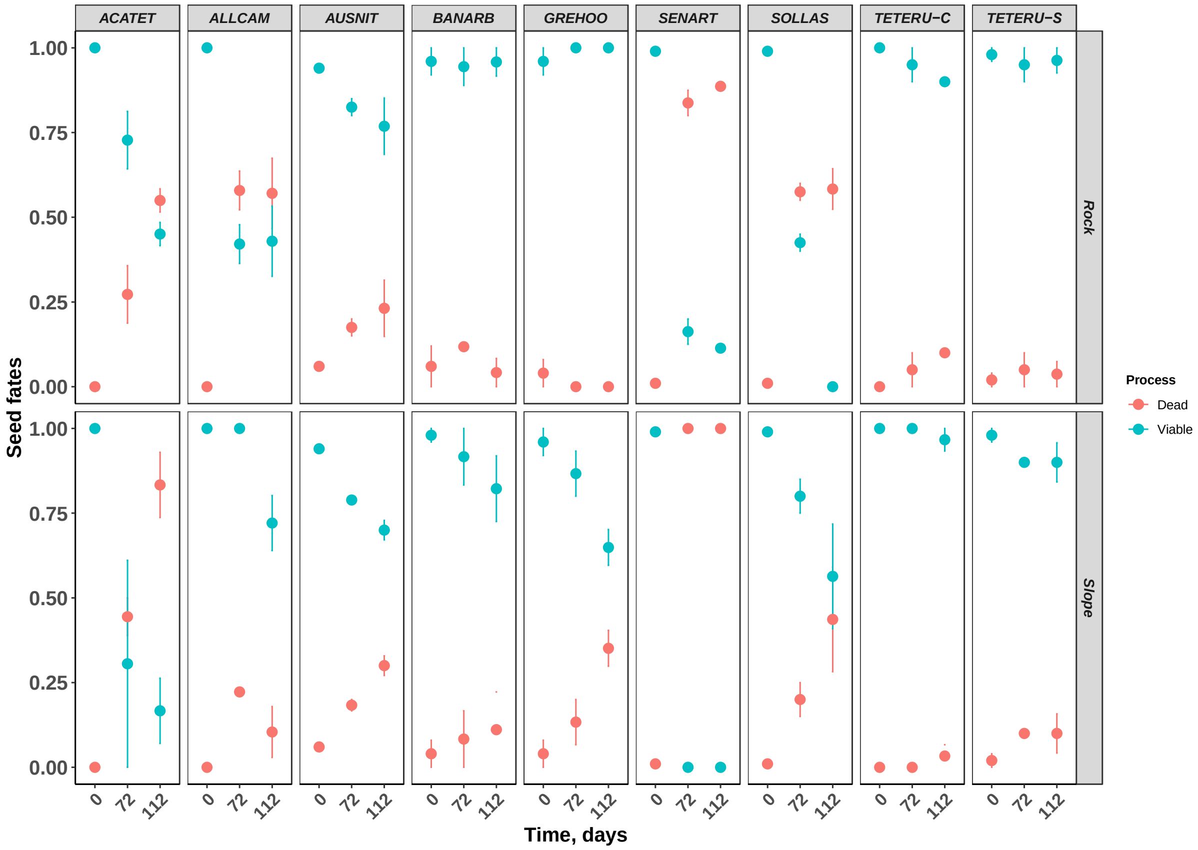


Figure 2.1.1f. Seed fates (persistence/viability; and dead seeds) after 112 days in rock and adjacent slope niche ecotypes in eight co-occurring species (*Acacia tetragonophylla* – *ACATET*, *Allocasuarina campestris* – *ALLCAM*, *Austrostipa nitida* – *AUSNIT*, *Banksia arborea* – *BANARB*, *Grevillea hookeriana* – *GREHOO*, *Senna artemisioides* subsp. *filifolia* – *SENART*, *Solanum lasiophyllum* – *SOLLAS*, and *Tetratheca erubescens* – *TETERU-C*; *TETERU-S*).

2.1.3 Determine the environmental requirements (crack attributes, aspect, temperature and moisture) for establishing plants *in situ*

Research outcomes:

- The environmental requirements for establishing plants *in situ* were determined by monitoring soil microclimate using high resolution soil moisture and temperature loggers, and characterising the habitat (substrate, position, vegetation) of the planting locations (one-off installation in 2017).
- Peak soil temperatures were up to 69°C in summer 2017/2018, and <60°C in 2018/2019, 2019/2020, and 2020/2021 summers.
- The hot summers in 2019/2020 and 2020/2021 were matched by prolonged periods without moisture recharge from rainfall.
- Baseline (*ex situ*) plant function responses to drought declined after three days and plants significantly deteriorate after ten days, indicating a functional threshold to reductions in soil moisture availability.
- The environmental requirements of greenstock planting locations that would more likely support survival appear to be a steeper, continuous local slope or a non-continuous local slope (a right-angled position); a large immediate local catchment area; and a smaller width to the planting area (or shelf).
- Several habitat characteristics were similar among all locations, regardless of whether it contained a live plant or not (water capturing or water shedding; rock substrate as opposed to rock-bench or ground substrate; or a similar average angle of aspect).
- Crack attributes (around planting location) or aspect did not appear to be key characteristics that indicated improved greenstock survival.
- There were too few seedlings established from *in situ* sown seed to characterise their environmental requirements effectively.
- **Conclusion:** Changing conditions (temperature and soil moisture) and planting location (habitat characteristics) strongly affect plant responses and these specific environmental requirements need to be considered when selecting translocation sites, planting locations and exploring potential field amendments (e.g. irrigation) to improve plant establishment. These research outcomes provide valuable information for the targeted placement of new planting and sowing locations at existing translocation sites (or new translocation sites) to increase survival outcomes under future installation opportunities.

Habitat characteristics

Review of planting locations

Greenstock planting locations that contained live plants in 2021 had several habitat characteristics that were different to those locations that had dead plants. The following statements are based on an estimated group average, and there were specific locations that varied from this average in either direction (higher or lower). See Annual Report 2 for definitions of habitat characteristic (Elliott *et al.* 2019).

- Planting locations of alive plants had a steeper, average angle of continuous local slope than locations of dead plants (alive = 34.4-50.4° or dead = 18.4-22.4°), when excluding locations that were in right angled positions. For locations that were right angled positions (i.e. a non-continuous slope angle), there were more of these with alive plants than those with dead plants (alive = 67.7% or dead = 49.7%).

- The immediate local catchment area was almost twice as large on average for locations with alive plants than those with dead plants (alive = 1131cm^2 or dead = 600cm^2).
- The average width of the immediate planting area (or shelf) was smaller at locations with alive plants than those with dead plants (alive = 13.9cm or dead = 21.1cm).

There were some habitat characteristics that were similar among all locations, regardless of whether they contained a live plant or not.

- The majority of locations were classified as water capturing (alive = 79% or dead = 75%), rather than water shedding.
- Most locations occurred in rock substrate (alive = 84% or dead = 60%), as opposed to rock-bench or ground substrate.
- Planting locations had a similar average angle of aspect (northern side = 109-124° or southern side 238-246°).
- Characterisation of the average crack width (7.7-13.1mm), depth (13.9-15.9mm) and distance to next nearest crack (12.6-14.7cm) were similar among planting locations.
- The average height of the "immediate local cliff" was similar among planting locations (22.4-25.1cm).

2.2 *Survival, growth and reproduction in restored and natural populations.*

2.2.1 Develop baseline data on the growth, survival, flowering and seed production of seedlings, juveniles and mature plants

Natural population

Research outcomes:

- Floral and fruiting phenology in 2021 differed to 2019 or 2020 but was similar to that observed for the 2018 season for adult plants.
- Growth rates of adult plants on the northern side were similar, relative to their size, in comparison to plants on the southern side, and the amount of growth observed was similar to that seen in 2018.
- Differences observed between these growing seasons may be partly explained by environmental conditions (rainfall, temperature), with both 2018 and 2021 having 30-40mm more rainfall than the annual average, while 2019 and 2020 had 20-80mm less rainfall than the annual average (294mm) for Koolyanobbing (Figure 2.2.1a).
- Overall, the demographic dynamics of *T. erubescens* have similar characteristics to other Goldfields *Tetratheca* species (described in Yates *et al.* 2008), such as pulse recruitment during winter, high seedling mortality likely due to climate and micro-habitat characteristics, low adult mortality and seasonal changes in adult plant size (or health) that indicates resilience of this mature life stage.
- Further details of research are in Annual Research Reports 1-4 (Elliott *et al.* 2018; 2019; 2020; 2021).

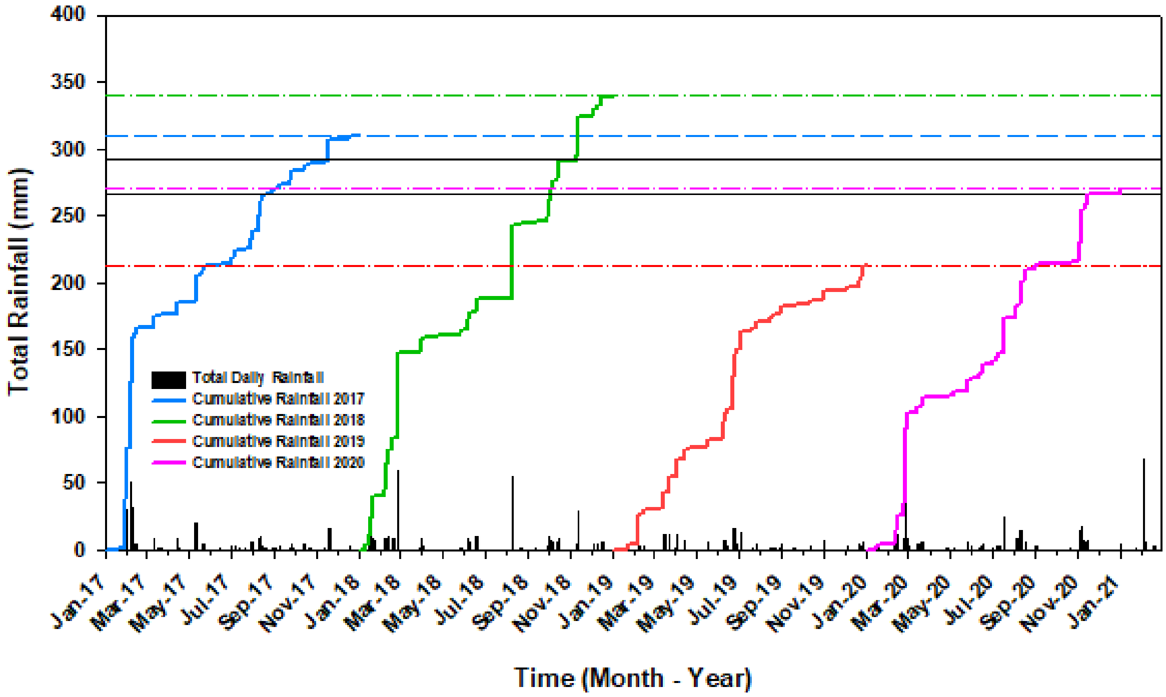


Figure 2.2.1a Cumulative rainfall for 2017 (blue line), 2018 (green line), 2019 (red line) and 2020 (purple line) between January 2017 and February 2021. The dashed horizontal lines indicate total yearly rainfall (2017: dashed blue line; 2018: dashed green line; 2019: dashed red line; and 2020: dashed purple line). Total rainfall over a period of 365 days is reported in Figure 2.1.3b. Rainfall data available from BOM, 2021 – Koolyanobbing, Site 12227, with the average total mean (bottom line) and medium (top line) rainfall represented as the black lines.

Translocated populations

Research outcomes:

- Greenstock survival patterns were similar amongst all translocation sites (within the same year).
- Greenstock survival patterns were different among each year's planting, with the best survival (after the first summer) occurring for the 2018 planting year (11.4%).
- 2021/2022 summer declines of *in situ* greenstock were greater in young greenstock (i.e. 20 months established) than older greenstock (i.e. 54 months established), which consistently supports previous results of the effect of greenstock age on likelihood of continued survival.
- Details are in Annual Research Reports 2-4 (Elliott *et al.* 2019; 2020; 2021).

Natural population

Floral and fruiting phenology

The average number of floral units (e.g. buds + flowers + fruit) in 2021 was higher than 2019 or 2020 (Figure 2.2.1b), and corresponds to the pattern of average winter rainfall that also occurred in 2018 (see Figure 2.2.1d in Elliott *et al.* 2020). The stage of floral development showed that the phenology of bud, flower or fruit production had similar responses between the years (i.e. skewed in one direction, and varied between the northern and southern sides;

Figure 2.2.1b). In summary, this data indicates that floral phenology is influenced by environmental conditions (e.g. aspect) and seasonal changes in rainfall or temperature among the different years.

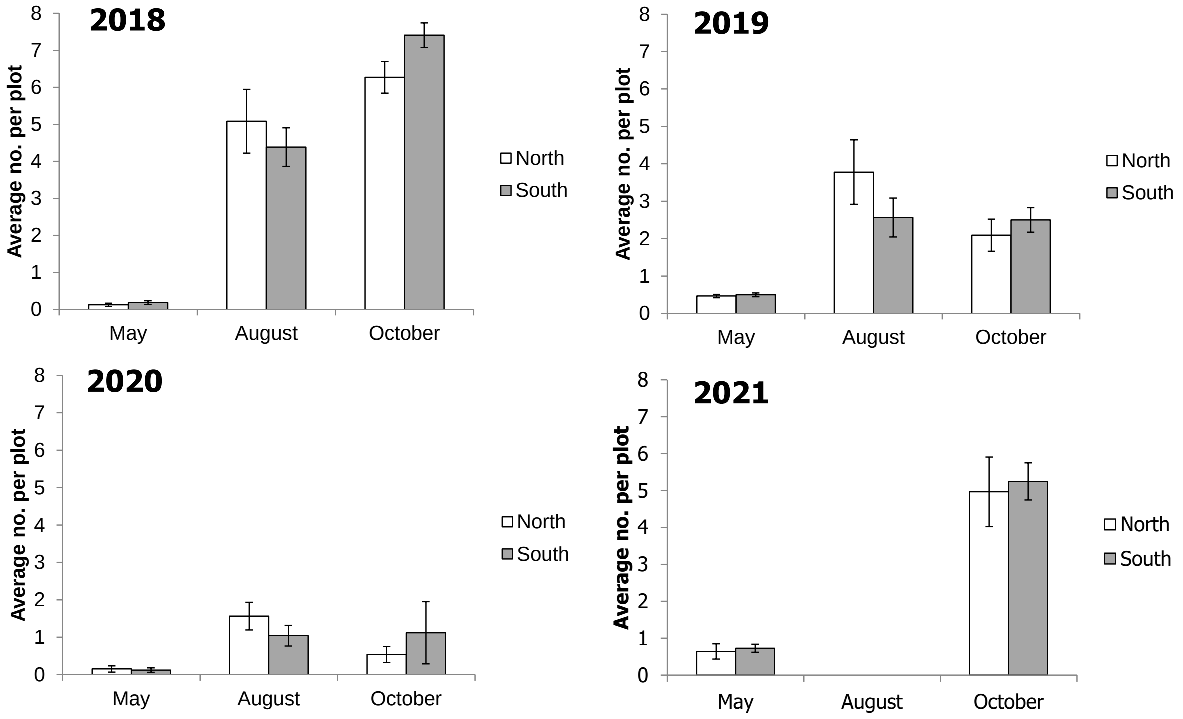


Figure 2.2.1b. Reproductive phenology (mean ± standard error) of plants (*n* = 20 per plot) in the natural population located on the northern or the southern side of the Koolyanobbing Range. Reproductive phenology represents the average number of floral units (e.g. buds + flowers + fruit) recorded for each month (average number of floral units per branch). Top left chart is the 2018 flowering season (Elliott *et al.* 2019); top right chart is the 2019 season (Elliott *et al.* 2020); the bottom left chart is the 2020 flowering season (Elliott *et al.* 2021); and the bottom right chart is the 2021 flowering season (August not measured).

Plant size and growth

The plant sizes and growth of adult plants in the natural population were assessed to establish the baseline physical attributes of plants. Adult plants on the northern side were smaller on average than those on the southern side (see Elliott *et al.* 2019 for details). New plant growth (new stems) of monitored plants occurred mainly between May and August (Figure 2.2.1c). Similar to 2020 patterns, plants on the southern side produced higher amounts of new growth, relative to their size, in comparison to plants on the northern side in 2021 (Figure 2.2.1c.a). Each plot performed similarly, with a greater proportion of new growth occurring in August (Figure 2.2.1c.b).

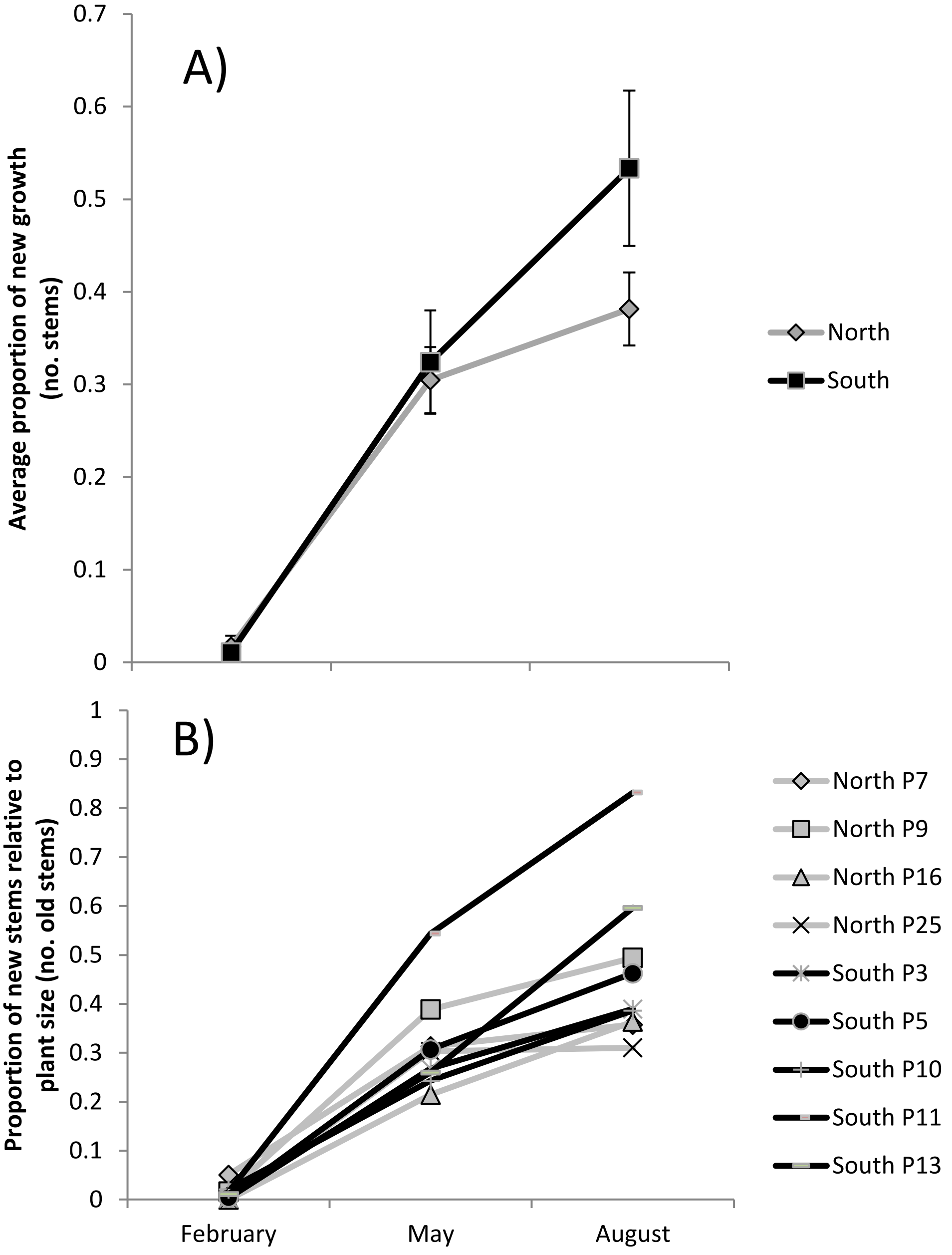


Figure 2.2.1c. Growth of adult plants in the natural population (*n* = 20 plants per plot), as measured by the number of new stems relative to the number of old stems on each plant over time (February – August 2021). A) overall plant growth (mean ± standard error) for the northern or southern side and B) growth rates (mean) within each individual plot, on the northern or southern side.

Overall plant growth in 2021 was 31-89% and most similar to the 60-70% observed in 2018 (Elliott *et al*. 2019; Elliott *et al*. 2020). Differences observed between these growing seasons may be partly explained by environmental conditions (rainfall, temperature). The premature growing season of 2020 (see Elliott *et al*. 2021) is most likely the reason for such variation in growth, as each plot recovered differently from this event, with plants on the northern side adding the least amount of relative growth.

Natural population recruitment

In October 2021, recruitment of *T. erubescens* seedlings occurred in some of the established monitoring plots in the natural population (*n* = 26 seedlings). At the time, seedlings and some juvenile plants were sensitive to assessments due to their low abundance, location accessibility and very small size, therefore, a limited number of measurements were taken to minimise any impact to their survival.

Translocated population

There was varied survival of greenstock between planting years and each planting year had survivors that experienced multiple summers.

Status of greenstock

- 2017 Translocation: Survival after 4.5 years (four summers) ranged from 0.4 – 4.4% per site, of the original planting (overall = 1.9% or 14 plants).
- 2018 Translocation: Survival after 3.5 years (three summers) ranged from 1.9 – 10% per site, of the original planting (overall = 4.4% or 48 plants).
- 2019 Translocation: Survival after 2.5 years (two summers) was extremely low, with an overall survival of 2 plants (0.4% of the original planting).
- 2020 Translocation: Survival after 1.5 years (one summer) ranged from 1.6 – 4% per site, of the original planting (overall = 2.2% or 16 plants).

Site differences

Translocation sites had similar survival of greenstock after each summer for each year's translocation (Wilcoxon test; 2017 (4 summers): *n* = 3, *P* = 0.25; 2018 (3 summers): *n* = 5, *P* = 0.063; 2019 (2 summers): *n* = 5, *P* = 0.181; 2020 (1 summer): *n* = 5, *P* = 0.1).

Survival after first summer

The survival of greenstock after their first summer differed between translocation years (ANOVA; F = 5.565, df = 3, *P* = 0.01), with the 2018 translocation (11.4%±3.1) having significantly different survival to 2019 (1.1%±0.5) and 2020 (3.2%±1.4) translocations (Tukey test *P* = <0.04; Part 1: Figure 2.2.1).

Survival after 12 months

If greenstock survived the 1st summer, there was a higher percentage of these plants that survived the 2nd summer (2017: 40.7%±19.1; 2018: 47.1%±2.2), 3rd summer (2017: 43.8%±15; 2018: 90%±8.2) and 4th summer (2017: 75%±15; Part 1: Figure 2.2.1).

Planting time

Late winter planting with below average winter rainfall can result in poorer survival, particularly after summer (i.e. 2017 vs 2018).

Rainfall patterns

Above average winter rainfall but below average spring rainfall (78%) can result in poorer survival, equivalent to a "late winter planting with below average winter rainfall" response in survival (i.e. 2017 vs 2019; Part 2: Figure 2.2.1a).

2.2.2 Develop understanding of the importance of spatiotemporal environmental factors that drive variation in these population parameters.

Research outcomes:

- Annual variation in winter rainfall likely influences the size of the recruitment pulse, with a higher recruitment pulse observed in years with higher rainfall (e.g. 2019), regardless of whether it occurred in natural or translocated population. This indicated a capacity for seeds in translocation sites to emerge in response to natural rainfall events at the same time and similar magnitude to natural recruitment, in relative terms only because the comparative number of seed in the soil seedbank in the natural population was unknown.
- Following recruitment events during the cooler wetter months (winter), the high mortality of these seedlings during the first summer (this response is the same annually; see Section 2.2.1 in each Annual Report 2018-2021) indicates the typical seasonal shift to increased temperature and decreased soil moisture after recruitment significantly impairs the survival capacity of young seedlings in most situations.
- The broad habitat niche for emergence of seedlings, whether in natural (crack or ground) or translocated (crack or drill hole) locations, and the survival of the limited few recruits to a narrower habitat niche (crack, but not all cracks) indicates that seedling establishment is more dependent on the location being able to support the establishment of a seedling (see Section 2.1.3 above) rather than the emergence of a seedling (see Section 1.1.2 above).
- Similarly, the patterns of growth, plant health (condition) and reproduction are influenced by climate and the environment (e.g. aspect). It is during the hotter and drier periods of the year where performance decreases, again regardless of whether it occurred in natural or translocated population (see Section 2.2.1 above), and if the survival threshold is maintained then performance shifts to a higher function during the cooler and wetter periods. Although the intensity of this higher function (i.e. number of new stems or flowers) depends on the average conditions of the climate that season. For example, the better performing plants occurred on the cooler southern aspect of Koolyanobbing Range and performed the best (e.g. increased growth and longer flowering window) during the cooler and wetter part of the year (winter; see Annual Reports 1-4 (2018-2021) or Part 2 of this report).

There was a positive correlation between the amount of rainfall and the number of seedlings that emerged in the translocation and natural population (corrected Spearman's Rank $P = 0.017$), that is, it was more likely to have more seedlings appear after higher winter rains (e.g. compare year 2017 with 2019; Figure 2.2.2a). Interestingly, the behaviour of seedling emergence followed the same pattern of response (high or low per year) for the natural population (Figure 2.2.2a.a) and the translocation sites (Figure 2.2.2a.b). This indicated a functional similarity in recruitment responses because the translocation sites were mirroring natural recruitment events in the environment. This strategy of pulse recruitment and variable mortality among the different life stages was also found for a sister species (*T. paynterae* subsp. *paynterae*; Yates *et al.* 2008; 2011), indicating the development of similar strategies for responding to the habitat and environmental conditions of banded ironstone that is experienced by sister species that are 100km apart.

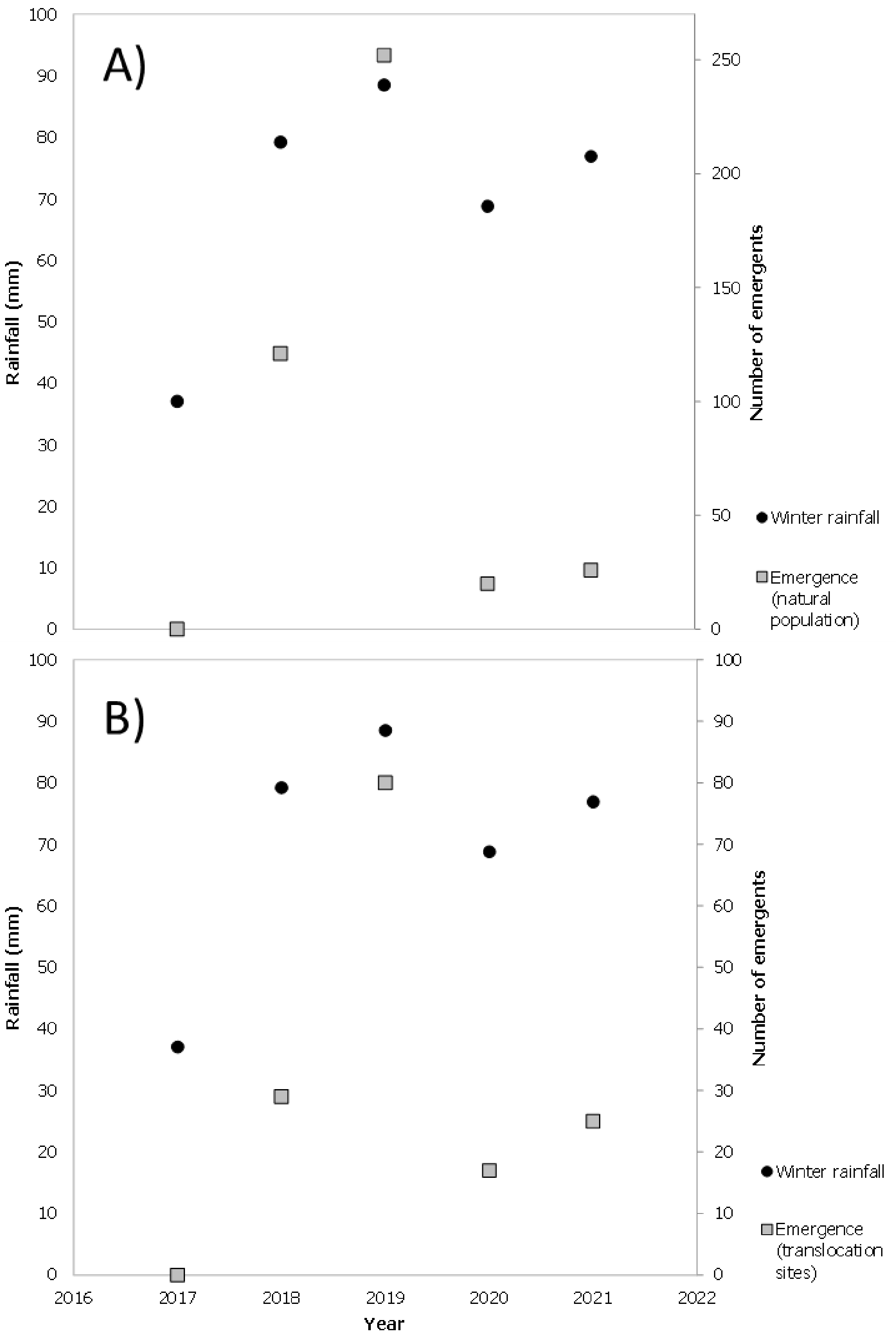


Figure 2.2.2a. The number of seedlings that emerged in the A) natural population and B) translocated population, plotted with the average winter rainfall (1 June - 10 August) that occurred for each year. Rainfall from BOM, 2021 (Koolyanobbing, station 12227).

2.2.3 Model the dynamics of *T. erubescens* populations to increase understanding of parameters such as expected longevity and time to maturity.

Research outcomes:

- An expanded conceptual model that linked the processes of developing a translocated population to the life cycle of a natural population of *T. erubescens*, demonstrated that the production and incorporation of seeds into the system was the key step to establishing a self-sustainable population.
- The life stage population model analyses, based on the transition and survival probabilities that characterised each one-year time step of the monitoring programs (2017-2021), predicted a decline in population growth rates of the translocated population and had mixed predictions (decline or growth) of population growth of the natural population. This is understandable considering the strong annual variation in all the baseline parameters measured in the translocated and natural populations (see Part 1: Table 2.2.1a and 2.2.1b).
- Caution needs to be applied when interpreting these predicted population growth rates. Consideration needs to be given to the limitations of generating a translocated population that is driven by significant mortality; the natural population was sub-sampled and individual variation was generalised as an average. Models are subject to data quality, and this sampling may or may not be adequate to derive some of the more sensitive parameters to understanding population dynamics. The strength of these population model analyses can only be improved with having data on additional translocation responses (i.e. new or improved translocation installations) and longer monitoring lengths (i.e. accurately capture transitions) incorporated into the models.
- Future translocation efforts and research needs to focus on increasing the size of the theoretical link between a translocated population and the life cycle of a natural population by overcoming the survival bottleneck of the translocated population so reproductive maturity is reached and the translocation can successfully transition it to a functioning natural population.

Background and method

Understanding population dynamics (births, deaths, life stage transitions) is vital to identifying the stages that are most critical to population growth and persistence. Long-term demographic data is required to derive parameters that are used in modelling to determine likely trajectories (stable, increasing, decreasing) of a population.

The first step to population modelling is to construct a conceptual model of the life cycle of the target organism. For *Tetratheca erubescens*, we used the conceptual model of the life cycle of a natural population of *T. paynterae* subsp. *paynterae*, as outlined by Yates *et al.* (2008), and expanded on it to include the translocated population. This conceptual model provides the structure for the development of the transition and survival matrices that were used in population model analysis.

The second step is to populate these matrices for the 1) translocated population using pooled data from the five years of translocation experimentation and monitoring (e.g. survival of greenstock or the transition of seeds to seedlings) and 2) natural population using pooled data from the five years of demographic monitoring (e.g. survival of seedlings or transition of juveniles to adults). These two types of matrices were then used in the population model analyses with data being pooled across sites and carried over monitoring years. The population model analyses used a simplistic model (i.e. proportions of survival and life stage transition) to increase our understanding of the parameters that strongly influence population dynamics. Determining a hypothetical population response under different scenarios (or years) provides valuable information that informs the likely sensitivities in the 1) translocation process or 2) natural population life cycle that may explain patterns observed in each.

The conceptual model

For the expanded conceptual model, we used propagation and biological classification to define the different stages of a translocated population (e.g. its development) and natural population (e.g. its life cycle). The conceptual model combined the knowledge gained from Program 2, on the different stages involved in the development of a translocated population using direct seeding and greenstock planting, and linking this to the different stages in the life cycle of a natural population (see Table 2.2.3a for definitions of each life stage). For each stage of the conceptual model (Figures 2.2.3a), one stage (e.g. juvenile) can transition to another stage (e.g. produces flowers and become a reproductive adult) or remain at the same stage of development (e.g. stay as a juvenile) or die. This conceptual model illustrates the life stage that theoretically connects translocated populations to the life cycle of a natural population – the production and incorporation of seeds into the system (Figure 2.2.3a).

Population model analysis

The monitoring data that supports the development of the two types of matrices used for the population modelling has been reported and described in Program 2 (see Annual Reports 1-4; Elliott *et al.* 2018-2021). The proportion of individuals in each stage that transitioned, remained or died were calculated for each of the four one-year time steps that were monitored in the translocated and natural populations (2017-2018; 2018-2019; 2019-2020; 2020-2021). The population growth rates from the transition and survival matrices were calculated using the PopTools add-in for Excel (Hood 2010).

The fecundity (or reproduction) estimates were calculated from the average number of seeds produced per stem per year (Section 2.2.1). For the translocated population, the model was run under two reproductive scenarios (f_{sdctr} in Figure 2.2.3a) that represented the minimal seed production numbers for young translocations and the average seed production numbers for older translocations from the translocation monitoring data, to obtain a range in response from the model for the translocated population. For the natural population, the model was run under four different reproductive scenarios (f_{sdR1} in Figure 2.2.3a) that represented the minimal, below average, average and above average seed production estimates from the demographic monitoring data, to obtain a range in response from the model for the translocated population. Data from the seed burial trial was used to estimate the amount of seed that remained alive (a_{sdsd} in Figure 2.2.3a) in the soil seedbank each year (data from T6 only: 85%; Part 2: Section 2.1.1) for both the translocated and natural population matrices. Data from the emergence lines was used to estimate the number of seed that transition into a seedling (data from T6

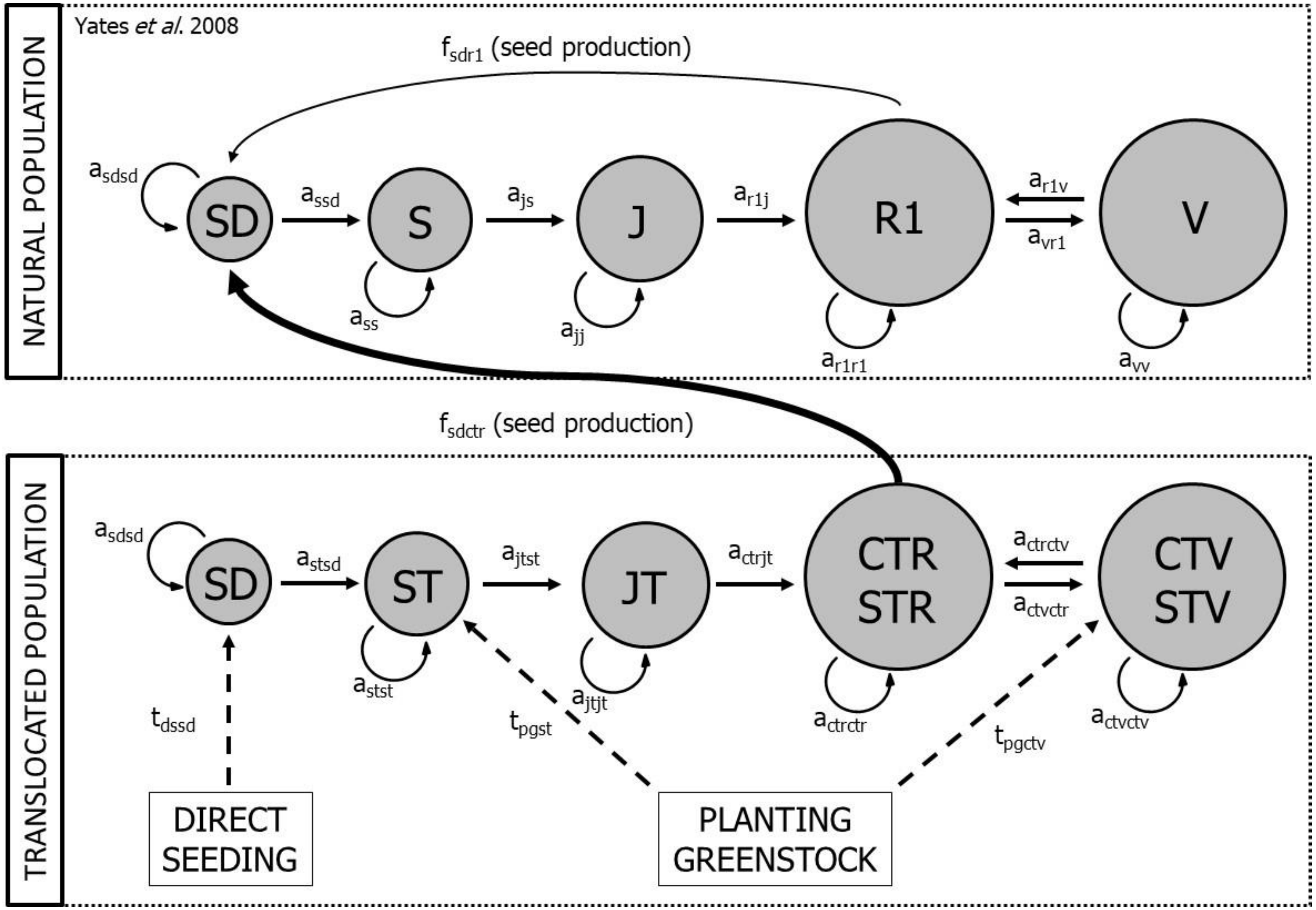


Figure 2.2.3a. We expand on the conceptual model of the life cycle outlined by Yates *et al*. (2008; top box), by linking the stages of developing a translocated population (bottom box) to the life cycle of a natural population of *Tetratheca erubescens*. The translocation actions (i.e. direct seeding, planting greenstock) initiate several stages of the translocation process and the transition between these stages culminates in the translocated population producing seed, which theoretically links the translocated population to the conventional life cycle of a natural population (thick arrow). Each circle represents a life stage in the natural or translocated population and each arrow represents the transitions (or change of stage) between life stages or the maintenance within that stage (see Table 2.2.3a for definitions of each stage).

only: 1%; Annual Reports 2018-2021: Section 2.1.1) for both the translocated (a_{ssd} in Figure 2.2.3a) and natural (a_{stsd} in Figure 2.2.3a) population matrices. These three estimates were held constant for each of the four one-year time step matrices for the translocated and natural populations. These estimates are likely to be an over-estimation in poorer production years (e.g. 2020) and an under-estimation in highly productive years (e.g. 2017), however, it represents overall averages across the monitoring period (five years) and provides a range in response of the populations from the model analysis.

Table 2.2.3a. Definitions of the variables contained in the expanded conceptual life cycle of *T. erubescens* that links the processes involved in developing a translocated population to the stages of the life cycle of a natural population (Figure 2.2.3a).

Figure codes	Variable	Definition
a		Survival and transition between, or within, a life stage
f	fecundity	The production of seed
t	translocation action	Sowing of seeds (ds) or planting of greenstock (pg)
SD	seed	Alive and in a dormant or non-dormant state
S	seedling	First year emergent or 2-3 years old (no adult foliage)
J	juvenile plant	Evidence of adult foliage (>110mm height)
R1	reproductive adult	Produces flowers and fruit
V	vegetative adult	No evidence of flowers or fruit
ST	seedling greenstock	Greenstock propagated from seed
JT	juvenile greenstock	Greenstock propagated from seed with evidence of adult foliage
STR	seed-derived greenstock – reproductive	Greenstock propagated from seed and produces flowers and fruit (mature)
STV	seed-derived greenstock – vegetative	Greenstock propagated from seed and no evidence of flowers or fruit (has previously)
CTR	cutting-derived greenstock - reproductive	Greenstock propagated from cuttings and produces flowers and fruit (mature)
CTV	cutting-derived greenstock - vegetative	Greenstock propagated from cuttings and no evidence of flowers or fruit (mature)

Results

Translocated population growth
The simplistic population model predicted intrinsic population growth rates of the translocated population based on the specific parameters that characterised each one-year time step of our monitoring program (Table 2.2.3b). There was a predicted decline in population growth based on the transition and survival probabilities observed in the translocated populations so far (2017-2021). This is understandable if one reflects on the baseline parameters measured in the translocations that are summarised in Table 2.2.1b (Part 1). The translocation process has not yet established a translocated population that meets the requirements of the current simplistic model (probabilities of transition, survival, or fecundity) to show population growth, and this is directly related to the data collected on translocation baseline parameters - the small amount of greenstock that were reproductive, survived or seedlings that transitioned to juveniles (i.e. none, as not long enough monitoring time). The only shift in this prediction (i.e. a smaller predicted decline in population growth) occurred for the last time step (2020-2021), indicating that parameters and conditions were different enough to generate a different model outcome. There was no difference between the reproductive scenarios (i.e. seed production) at any time step, which indicated that this level of translocation fecundity (4-46 seeds/plant) did not strongly influence model outcomes.

Interestingly, the major differences between the time steps can be characterised by:

- 2017-2018; 2018-2019; and 2019-2020: lowest survival probabilities and number of greenstock that were reproductive.
- 2020-2021: highest survival probabilities and number of greenstock that were reproductive.

Table 2.2.3b. Predicted rate of intrinsic population growth (λ as a %) for each of the four one-year time steps of monitoring in the translocated population under two different reproductive scenarios (minimal seed production numbers for young translocations and average seed production numbers for older translocations, 4.5 years old).

One-year time step	Minimal (4 seed/plant)	Average (46 seed/plant)
2017-2018	- 15%	- 15%
2018-2019	- 15%	- 15%
2019-2020	- 15%	- 15%
2020-2021	- 8.7%	- 8.7%

Even though the models were resolved, caution needs to be applied when interpreting these predicted population growth rates, as translocated populations are an artificial beginning to generating a population that is driven by significant mortality, and this encompasses different and varied assumptions to consider when viewing these results (e.g. maturity, time, established versus initiated populations, origin). The strength of these population model analyses can only be improved with having data on additional translocation responses (i.e. new or improved translocation installations) and longer monitoring lengths (i.e. accurately capture transitions) incorporated into the models.

Natural population growth

The simplistic population model predicted intrinsic population growth rates of the natural population based on the specific parameters that characterised each one-year time step of our monitoring program (Table 2.2.3b). There was a mixed response in the prediction of the models for intrinsic population growth based on the transition and survival probabilities observed in the natural populations so far (2017-2021). This is understandable if one recalls the strong annual variation in all the baseline parameters measured in the natural population that are summarised in Table 2.2.1a (Part 1) and all previous reports (see Annual Reports 1-4; 2018-2021). For example, the conditions (e.g. environmental) of the 2018-2019 time step had certain probabilities of transition and survival that generated models of predicted population growth under higher seed production scenarios and population decline under lower seed production scenarios. In contrast, the conditions of the 2020-2021 time step generated models of predicted population growth under all seed production scenarios. With such variation in model outcomes, the results here should be considered exploratory and not definitive (Yates *et al*. 2008).

Interestingly, the major differences between the time steps can be characterised by:

- 2017-2018: no recruitment and the transition matrix was constrained due to it being the first year of monitoring, so caution required with interpretation.
- 2018-2019: moderate recruitment with low seedling survival and a moderate number of reproductive adults.
- 2019-2020: few mature plants being reproductive and higher recruitment but very low seedling survival.
- 2020-2021: a high number of mature plants being reproductive and having both juveniles and seedlings having high survival rates (i.e. strong survival probabilities).

Table 2.2.3b. Predicted rate of intrinsic population growth (λ as a %) for each of the four one-year time steps of monitoring in the natural population under four different reproductive scenarios (minimal ~2020 seed production numbers and above average ~2017 seed production numbers).

One-year time step	Minimal (8 seed/plant)	Below average (50 seed/plant)	Average (80 seed/plant)	Above average (110 seed/plant)
2017-2018	0 %	0 %	0 %	0 %
2018-2019	- 8.0%	- 1.1%	+ 1.6%	+ 3.6%
2019-2020	- 0.1%	+ 0.4%	+ 0.7%	+ 0.96%
2020-2021	+ 8.2%	+ 21.9%	+ 27.0%	+ 31.0%

Caution needs to be applied when interpreting these predicted population growth rates, as only the north-western part of the natural population was able to be demographically monitored at Koolyanobbing Range (37%; nine out of 26 possible plots); and there was much unique variation in the number of seed that could be produced by an individual due to its location or size becomes generalised when using averages in model analyses.

In summary, the range of responses observed in the model outcomes demonstrate the difficulty in predicting the overall long-term trajectory of a population (growth, stability or decline) because there are many parameters that influence population dynamics. However, the outcomes of the population models do indicate that the natural population may experience bottlenecks in population growth at the stages of seedling survival, whilst the adult stages were more influential on population dynamics through the input of seed into the system because they had such high probabilities of survival. Changes in the survival or transition of these stages would strongly influence population dynamics of *T. erubescens*, and this concurs with the population viability analysis conducted by Yates *et al.* (2008), which concluded that any increase in reproductive adult mortality of *T. paynterae* subsp. *paynterae* would be a cause for concern.

Functional translocated population

The expanded conceptual model that linked the processes of developing a translocated population to the life cycle of a natural population of *T. erubescens*, demonstrated that the production and incorporation of seeds into the system (f_{sdctr} in Figure 2.2.3a) was the key step to establishing a self-sustainable population. To improve this probability in the model, the translocation process needs to ensure higher survival of reproductive greenstock in translocated populations to initiate a stronger connection to the life cycle of a natural population. The current translocations did establish this theoretical connection with the life cycle of the natural population, with evidence of greenstock producing fruit and seed. However, the quality of seed produced and its incorporation into the soil seedbank was unconfirmed and based on the model outcomes, most likely not large enough to secure a link with the functions of a natural population life cycle. Therefore, future translocation efforts and research needs to focus on increasing the size of this link and overcome the survival bottleneck of the translocated population so it can successfully transition it to a functioning natural population.

2.2.4 Compare performance of plants (growth, survival, flowering and seed production) in natural and translocated sites.

Research outcomes:

- Survival of translocated greenstock (2.8%) was lower overall, than the survival of monitored adult plants in the natural population (99.4%).
- Growth of greenstock was generally higher (39-163% relative to plant size) than the growth of the natural plants (20-71% relative to plant size).
- The difference in survival and growth between these groups of plants is due to their stage of life, with adult plants in the natural population at a stable phase (i.e. already established), while seedling and greenstock plants are transitioning between phases (i.e. vulnerable pre-establishment stages as emerged seedling or newly planted greenstock, to older and more mature stages).
- As with previous years, the quantity of flowering and seeding of greenstock plants was lower than the more established natural adult plants.

The comparative performance of mature plants in the natural population to the greenstock of the 2017 (4.5 years), 2018 (3.5 years), 2019 (2.5 years) or 2020 (1.5 months) translocation plants was difficult to make for some measures due to the young age and poor survival of greenstock. For example, survival or growth of adults was not a realistic comparison to make to greenstock, as almost all monitored natural plants remained alive for the duration of the monitoring unlike the translocated greenstock, where significant mortality was recorded after their first summer. However, those greenstock that did **survive** had higher chance of surviving the following summers (Table 2.2.4a). This shift in survival success after the first summer, was similar to the pattern observed for the naturally recruited seedlings (natural or translocated population), with these seedlings also having a low chance of surviving their first summer, but a higher chance of surviving the following summers (Table 2.2.4a). The difference in survival between these groups of plants is due to their stage of life, with adult plants in the natural population at a stable phase (i.e. already established), while seedling and greenstock plants are transitioning between phases (i.e. vulnerable pre-establishment stages as emerged seedling or newly planted greenstock, to older and more mature stages).

Greenstock **growth** was higher in some cases than the mature plants in the natural population, most likely due to the relatively smaller size of the greenstock in comparison to the mature plants (Table 2.2.4a). Interestingly, this relatively smaller size and higher growth was also observed for the naturally recruited seedlings and indicated access to resources that supported plant growth for newly establishing seedlings and greenstock.

The **plant condition** (% of dead biomass) of greenstock was closer in magnitude to that of the mature plants than the seedlings, with the mature plants having the greatest average decline in plant condition (Table 2.2.4a). This may indicate that cutting derived greenstock has similar responses as mature plants due to this being the source of propagation tissue for greenstock (i.e. inherited response) or that the summer conditions of the translocation site or planting location cannot support all the biomass grown on the greenstock during winter (i.e.

environmental response). A larger number of seedling derived greenstock would need to be planted to investigate the likely cause of this difference.

On average, greenstock had the poorest performance of **plant function** (as measured by chlorophyll florescence) of the three and this was prominent during summer, which may have reflected a higher environmental stress experienced by greenstock compared to adults and seedlings in the natural population (Table 2.2.4a). Interestingly, the seedling derived greenstock showed the poorest chlorophyll performance in spring. These spring measures may have been influenced by the late and below average rainfall recorded for 2019 and 2020 (Part 1: Figure 2.2.1) and indicate a greater sensitivity of seedling derived greenstock to these types of conditions than cutting derived greenstock.

Flowering and **fruit** production was quantifiable for older translocated plants. Although not directly comparable to adults in the natural population (i.e. size, maturity etc.), greenstock produced flowers and green or dehisced fruit (full sized) that indicated a capacity for greenstock to become reproductive within the first spring or at two years and be visited by pollinators at translocation sites. However, seed could not be collected as the plants were too fragile to place organza bags on them and longer-term studies need to determine seed production baselines for greenstock. Also a longer-time frame (>3 years) and larger number of seedlings that emerge from directly sown seeds will be needed to accurately determine the age to reproduction and the reproductive capacity of these seedlings in translocated sites.

Table 2.2.4a. Comparison between natural and translocated populations on the growth, survival, flowering and seed production of seedlings and mature plants. The overall average for the propagule is presented, with the range in variation presented in parenthesis, which captures annual variation, and differences between plants on the northern and southern aspects. See Annual Reports 1-4 (2018-2021).

	Mature Adult (natural population)	**Greenstock** (translocated population)		**Seedling** (natural population)	**Seedling** (translocated population)
Parameters		Cutting & Seedling		Soil seedbank	Sown seed
Monitoring period	4 years	>4 years		3 years	3 years
No. plants monitored	182	3,072		401	151
Plant age	Unknown	Un/Known		Known	Known
Age of reproduction	-	1st spring or 2 years		3 years	Unknown
Plants reproductive at start of study	Yes	Yes		No	No
Percentage survived (overall at end of 2021)	99.4%	2.8%		7.7%	0.8%[¥]
after 1St summer	-	5.4% (1.2 – 11.4%)		13.4% (5.5 - 20.5%)	3.2%
Of the survivors after 2nd summer	-	49.8% (25.0 – 90.0%)		70.4% (48.0 – 92.8%)	25.0%
Of the survivors after 3rd summer	-	78.1% (50.0 – 100%)		75.0%	100%
Of the survivors after 4th summer	-	100%		-	-
Percentage reproductive (of the total alive)	>90.0%	35.4% (10.1 – 58.8%)		22.2%	0%[§]
Percentage produced fruit (of those reproductive)	>91.6%	23.4% (1.3 – 53%)		1 of 2 seedlings	-
Average seed production	1-2 seed/fruit[^] 5-11 seed/stem[&]	Unknown*		Unknown*	-
Average growth (% new stems per year)	57.0% (38.1 – 71%)	104% (39 – 550%)		90.0% (50 – 130%)	-
Average plant condition (% plant brown)	26.3% (21.0 – 35.6%) spring 23.6% (18.3 – 54.5%) summer	15.5% (4.6 – 25.4%) spring 27.9% (20.9 – 42.1%) summer		10.4% (4.8 – 12.2%) spring 11% (5.6 – 14.1%) summer	-
Average chlorophyll performance (Fv/Fm)					
Summer	0.712	0.662		0.729	-
Autumn	0.740	0.739		0.738	-
Winter	0.769	0.758		0.706	-
Spring	0.709	0.714		0.745	-

[¥] Excludes 2021 emergents.

[§] Only one seedling survived multiple summers and therefore we could not gather data on the remaining parameters.

* Plants too small-fragile to passively collect potential seed by attaching an organza bag.

Program 3. Plant function, condition and water usage

3.1 *Plant function, condition and water usage*

3.1.1 Develop baseline data on the physiology and function of *T. erubescens* plants at seedling, juvenile and adult stages in natural populations

Research outcomes:

- As with previous years, the ecophysiological performance was similar between juvenile and adult plants in the natural population.
- Plant condition, as measured by the proportion of a plant that had recently died, was at its smallest proportion during the peak growing season (May-Aug) and largest during summer (Feb). This has been consistent across years (2018-2021).
- Each plot responded similarly, with decreases in the proportion of a plant that was dead from February to October 2021, indicating increased relative plant condition (on both sides of the ridge).

Plant health, measured by assessing chlorophyll fluorescence (Fv/Fm) on dark adapted leaves on plants in the natural population during early winter (June 2021) and mid spring (October 2021) showed that juvenile plants performed to a similar or higher level as that of adult plants (i.e. on the same side of the range; Figure 3.1.1a). This was consistent with the performance of juveniles and adults in previous years (Elliott *et al.* 2019-2021).

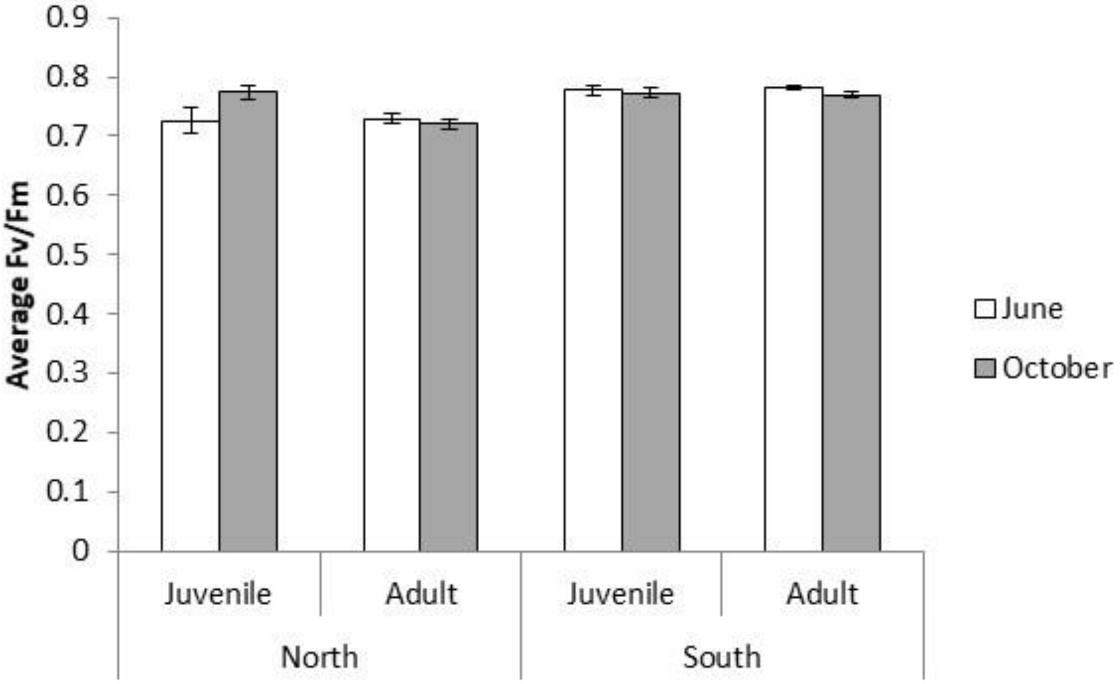


Figure 3.1.1a. Average chlorophyll florescence (Fv/Fm) measurements (mean ± standard error) of adult plants (*n* = 56-96 plants) and juveniles (*n* = 3-8 plants) in the natural population during two periods in 2021.

The peak condition of monitored plants (i.e. majority of the plant was green) occurred mainly during winter (see Elliott *et al*. 2019). In 2020, there was a steady decline in relative plant condition (i.e. proportion of the plant that was brown) from summer 2020, through the growing season (May-Aug) and into the following summer 2021. This pattern was similar for the northern and southern sides of the ridge (Figure 3.1.1b.a; Elliott *et al*. 2021).

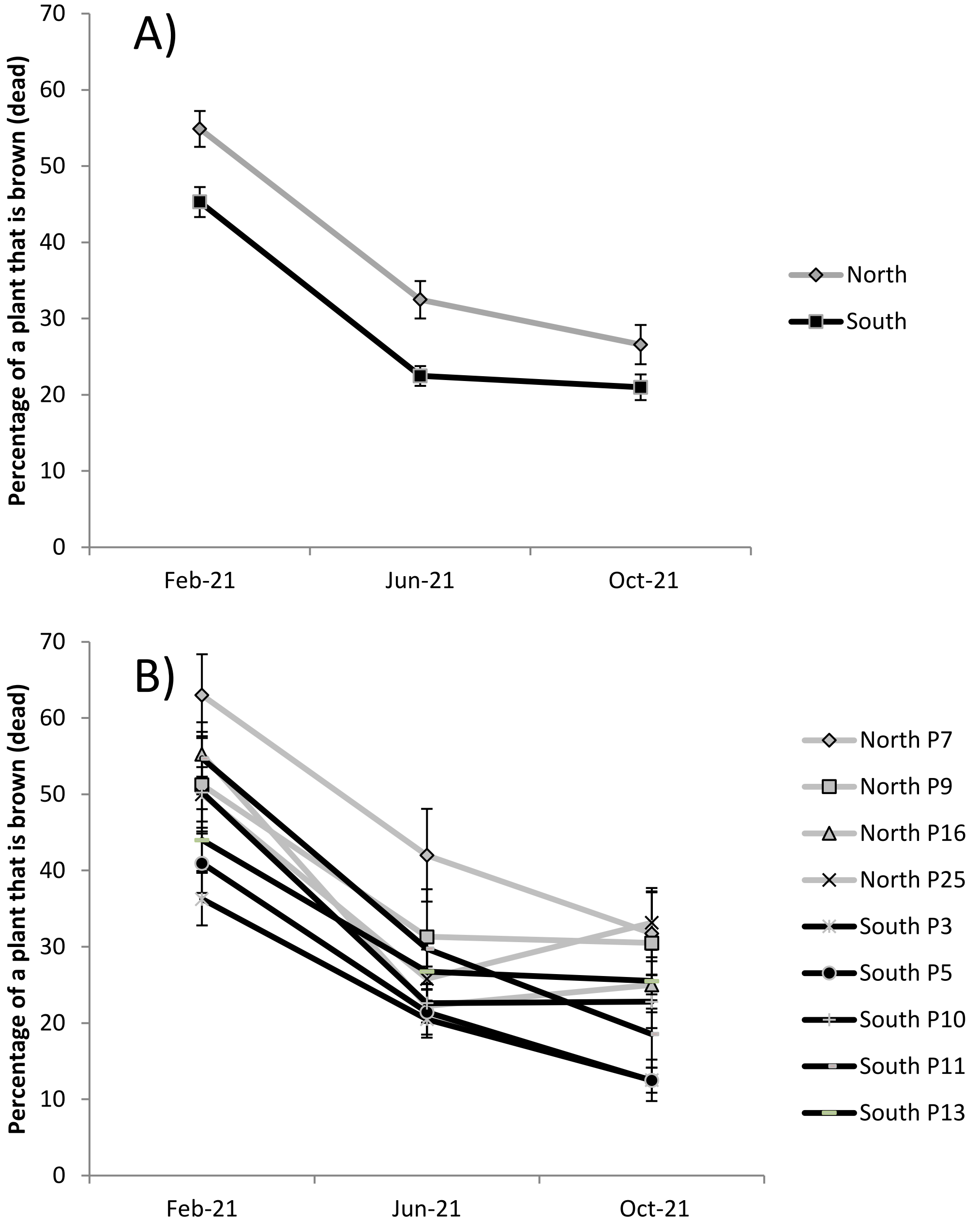


Figure 3.1.1b. Condition (mean ± standard error) of adult plants (*n* = 20 per plot) in the natural population, as measured by the percentage of an adult plant that was brown (i.e. newly dead plant tissue less than six months is a rich brown, not faded grey/white) over this time period (February 2021 – October 2021). A) overall plant condition averages (% plant brown) for the northern or southern side and B) average condition of plants within each individual plot, on the northern or southern side.

Plant condition of those monitored in the natural population, as measured by the percentage of the plant biomass that had recently died (% brown), had changed when comparing February 2020 with February 2021 (Figure 3.1.1b.b; Elliott *et al.* 2021). Unlike the previous three summers, where less than 1% of monitored plants had an observed plant condition of >90% brown biomass, in 2021 this increased to 5.5% of monitored plants with a recorded plant condition of >90% brown biomass (see Elliott *et al.* 2021). Such a change in relative plant condition between years has not been previously observed and was cause for concern.

However, the recovery of plant biomass after summer 2021 (Figure 3.1.1b) was observed, with plants having an increase in condition (i.e. lower % brown on plant) over the winter and spring seasons. The amount of biomass that was brown had dropped to levels (20-32% brown) observed in the growing season of 2020 (see Elliott *et al.* 2021). This indicated that plants were able to recover from the biomass lost during the 2021 summer.

3.1.2 Assess the impact of spatiotemporal variation in the environment (years, seasons, sites, habitat characteristics) on plant function

Research outcomes:

- Knowledge of how plants perform physiologically is key to understanding environmental effects on plant performance, productivity and survival. Seasonal ecophysiological measurements were conducted across 13 monitoring seasons, over three years between Summer, 2018 and Summer 2021.
- Our data demonstrates variation in ecophysiological performance based on stomatal conductance that was strongly driven by monitoring seasons across years, the aspect of the plots and differences between species
- By measuring spatiotemporal variation in plant performance and linking environmental variables against performance, we have identified sites on the Koolyanobbing Range that can cause increased plant stress. Additionally, the patterns measured over time and space provide a critical baseline information underpinning population performance.

Background and methods

Knowledge of how plants perform physiologically is key to understanding environmental effects on plant performance, productivity and survival; and is critical for *in situ* management actions such as site selection or identifying periods where plants are experiencing stress or active growth stages. We measured stomatal conductance – a measure of stomatal opening in plant leaves which can be used an indicator for plant water status; leaf temperatures; chlorophyll fluorescence – a measure for photosynthetic activity which can be used to assess levels of plant stress; and pre-dawn leaf water potentials – a measure of plant water status that assesses plant water stress in terms of the availability of water to the plant. In order to determine spatiotemporal variation in plant performance, seasonal ecophysiological measurements were conducted across 13 monitoring seasons, over three years between Summer, 2018 and Summer 2021 (except for pre-dawn leaf water potential: two monitoring seasons only). The ecophysiological responses were related to leaf temperatures, soil temperatures, rainfall, soil moisture and pre-dawn water potentials across two sites with a north-eastern (NE; Plots 7 and 25) and south-western (SW; Plots 3 and 5) aspect.

Ecophysiological performance of *T. erubescens* (*n* = 16-24 plants per aspect) was measured against two co-occurring species in the same niche: *Banksia arborea* (*n* = 4-6 plants per aspect) and *Eremophila decipiens* (*n* = 8-12 plants per aspect).

Results and conclusions

There was strong seasonal variation in plant performance driven by monitoring seasons across years (GLMM: $F_{12,1975} = 62.441$, $P < 0.001$), with winter periods presenting highest ecophysiological responses for stomatal conductance, and decreases of 33-51% as seasons transition into warmer summer periods (Figure 3.1.2a). There were differences in plant performance based on the aspect of the plots (GLMM: $F_{1,1975} = 92.361$, $P < 0.001$), with plants in NE-facing plots (Plots 7 and 25) presenting on average 20-30% decreases in functioning compared to SW-facing plots (Plots 3 and 5). Despite the differences in aspect, species generally demonstrated different patterns in responses (GLMM: $F_{2,1975} = 25.881$, $P < 0.001$), with *T. erubescens* and *B. arborea* generally performing poorer on NE-facing plots, whilst in *E. decipiens*, ecophysiological function was similar in NE and SW plots. The difference observed between species is likely a result of *E. decipiens* occurring across a greater breadth of ecotypes (and demonstrates a wider thermal range for ecophysiological function).

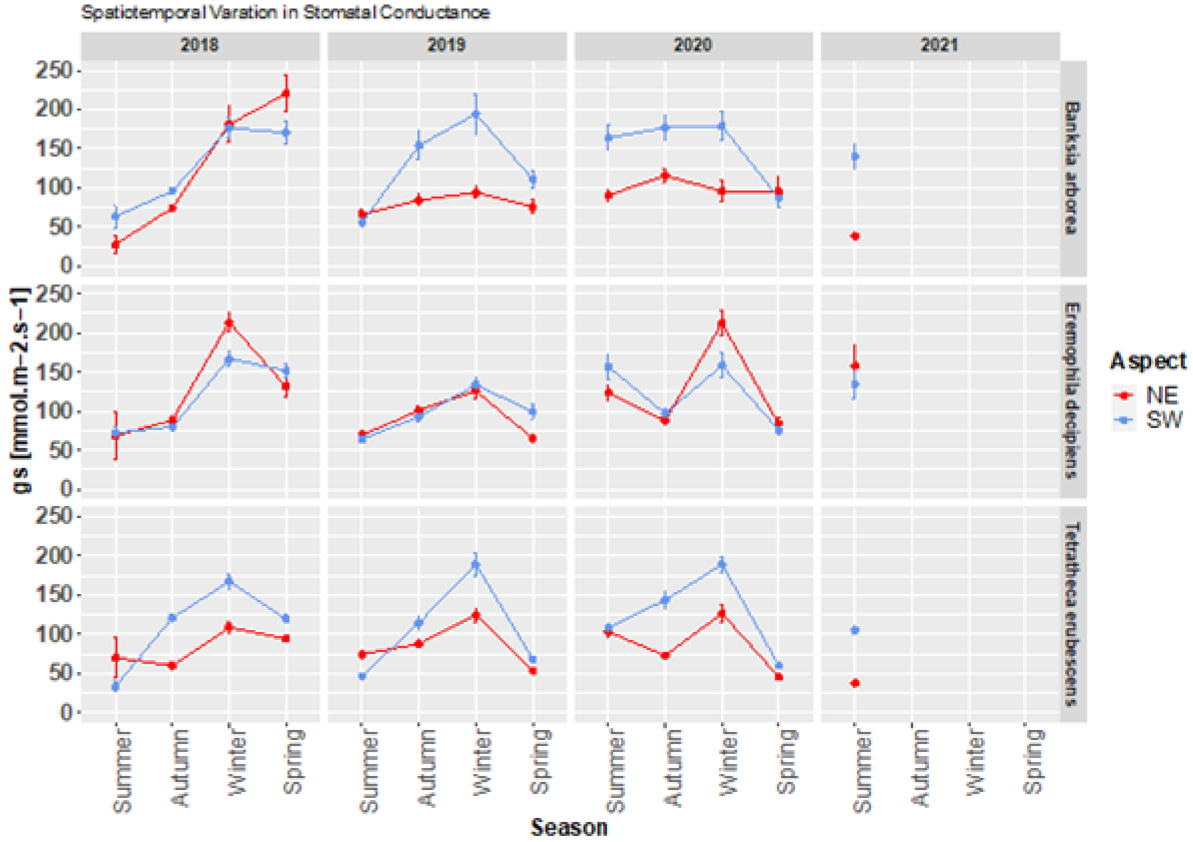


Figure 3.1.2a. Stomatal conductance (gs, mean ± standard error) of *Tetratheca erubescens* (*n* = 16-24) and two common BIF species (*Banksia arborea*, *n* = 4-6; and *Eremophila decipiens*, *n* = 8-12) in NE-facing (P7 and P25) and SW-facing (P3 and P5) plots.

Leaf temperatures were consistently higher for all species in NE-plots than in SW-plots (Figure 3.1.2b). Higher leaf temperatures were matched by lower stomatal conductance – indicating summer to be periods of decreased water-use and ecophysiological function for all species. For all species, leaf temperatures in spring 2019 were similar to leaf temperatures measured in the previous two summer periods, indicating a hot and dry spring period in 2019, had elicited a summer response in plants. These conditions are likely contributing to the decreased stomatal conductance measurements reported in spring 2019 (Figure 3.1.2a). The spring 2020 leaf temperature measurements were elevated for all species compared to the previous years and varied between 30-34°C. For all species, the elevated leaf temperatures in spring were similar to the leaf temperatures in the recent 2021 summer measurements. Taken together, these values indicate that plants were experiencing a prolonged period of heat across the landscape.

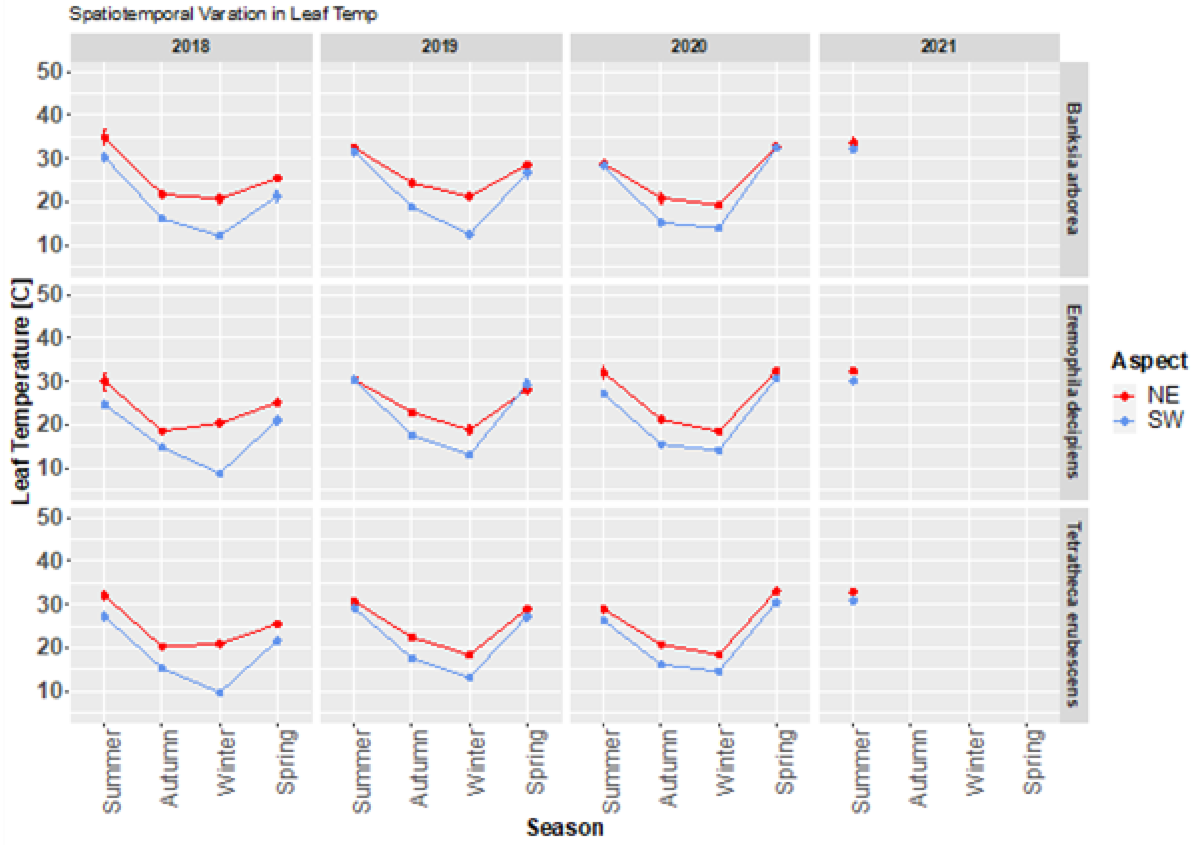


Figure 3.1.2b. Leaf temperatures (mean ± standard error) of *Tetratheca erubescens* (*n* = 16-24) and two common BIF species (*Banksia arborea*, *n* = 4-6; and *Eremophila decipiens*, *n* = 8-12) in NE-facing (P7 and P25) and SW-facing (P3 and P5) plots.

Chlorophyll fluorescence generally declined between spring and summer periods, with recovery observed between autumn and winter. For all species, lower chlorophyll fluorescence (Figure 3.1.2c) coincided with higher leaf temperatures in NE-facing plots (Figure 3.1.2b). Further corroborating the low stomatal conductance and high leaf temperatures measured during summer, low chlorophyll fluorescence values during summer indicate declines in stem health. The autumn 2020 measurements for *T. erubescens* were lowest on the NE-aspect (Fv/Fm

<0.6). These measures coincided with decreased stomatal conductance, which could have been driven by the decreased soil moisture available between March and May 2020 (see Figure 2.1.3a; P7 and P25). The increased chlorophyll fluorescence measurements in summer 2020 and 2021, demonstrate recovery following the spring senescence (Figure 3.1.2c).

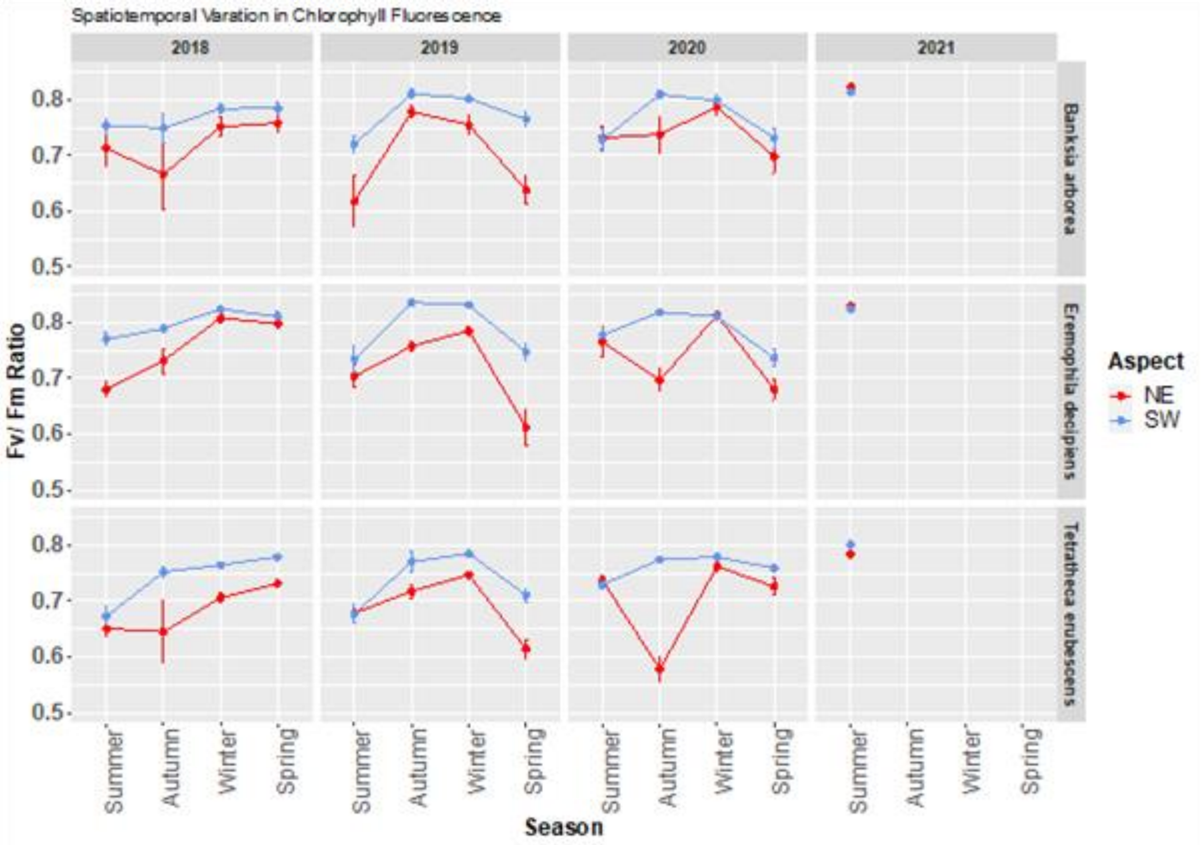


Figure 3.1.2c. Chlorophyll fluorescence (mean ± standard error) of *Tetratheca erubescens* (*n* = 16-24) and two common BIF species (*Banksia arborea*, *n* = 4-6; and *Eremophila decipiens*, *n* = 8-12) in NE-facing (P7 and P25) and SW-facing (P3 and P5) plots.

Given the seasonal trends in plant performance, measurements for pre-dawn leaf water potentials were conducted in the winter of 2020 (monitoring period 11) and summer of 2021 (monitoring period 13) to quantify plant available water in the landscape. Pre-dawn measurements indicate plant available water declining on average by at least 50% in summer in all species (Figure 3.1.2d). Together with decreased stomatal conductance and increased leaf temperatures presented by species during summer periods, the lower plant available water further corroborates summer as a high stress period. During winter, there was no difference in plant available water between NE- and SW-facing plots for *B. arborea* and *E. decipiens*, while SW-facing plots generally presented at least 20% higher plant available water than NE-facing plots in *T. erubescens*. Summer periods demonstrated strongest declines for all species, with aspect differences presented for *B. arborea* and *T. erubescens* but not in *E. decipiens*. These differences could be explained by the distribution of *B. arborea* and *T. erubescens* plants on the rock experiencing higher decreases in plant available water in summer due to higher temperature conditions on NE-facing plots, whilst *E. decipiens* plants were more evenly disturbed across non-rocky substrates in all sites.

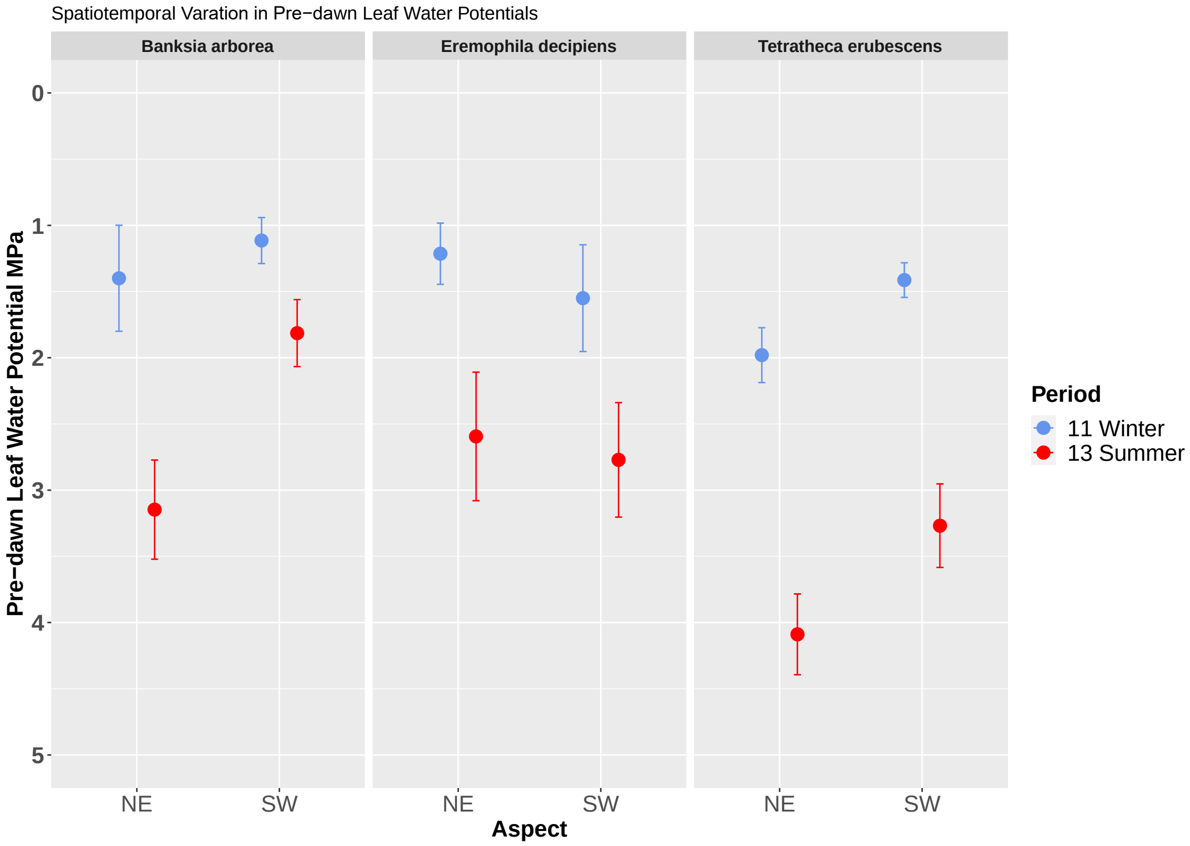


Figure 3.1.2d. Pre-dawn leaf water potentials (mean ± standard error) of *Tetratheca erubescens* (*n* = 16-24) and two common BIF species (*Banksia arborea*, *n* = 4-6; and *Eremophila decipiens*, *n* = 8-12) in NE-facing (P7 and P25) and SW-facing (P3 and P5) plots.

In conclusion, the data demonstrates NE-facing plots to not only be hotter, but also drier in terms of water availability for *T. erubescens*, with the plants experiencing higher water stress during summer periods than during winter. These conditions represent high stress periods that could have implications on plant survival in newly recruited seedlings, or for translocated plants on this aspect of the Koolyanobbing Range.

3.1.3 Identify the ecophysiological strategies employed by plants that enable them to survive and grow in rock fissures in a semi-arid environment

Research outcomes:

- A rock excavation trial was conducted in June 2017, whereby root architecture of *T. erubescens* plants were exposed. Roots were observed up to 1.5m into the rock substrate, indicating the capacity for *T. erubescens* roots to use the rock strata, along fissures, rock cracks and/ or through shallow soil channels.
- Genetic assessment determined that *T. erubescens* roots occurred in the rock profile to a depth of 400cm from the crest, indicating that the plants could exploit the cracks, fissures and cavities of the rock strata to locate potential pockets of accessible moisture.

Root network in rock substrate

In July 2017, an opportunity to assess and excavate plants in Stage 1 (SE) enabled observations to quantify the extent to which roots were distributed in the rock substrate, and inform on the possible ecophysiological strategies employed by *T. erubescens* (Elliott *et al.* 2018).

Plant roots exposed during the excavation of the rock profile were sampled and genetically assessed to identify if they were *T. erubescens* roots. Samples were collected at 11 locations from the rock crest (0cm) to the deepest accessible part in the profile (400cm). The total number of field samples collected through the rock profile was 21, as some locations (i.e. depths) had replicate samples collected. In addition, root samples were collected from under five *T. erubescens* plants that were on the rock. Root samples were collected, dried and stored on silica until processing.

Root samples (and known reference samples) were processed by extracting DNA from the sample using a modified Carlson DNA extraction method (Carlson *et al.* 1991). Samples ranged in size from thick diameter roots (3mm) to very fine hair-like roots (<0.5mm; Figure 3.1.3a). Depending on the amount of material available, some samples were sub-sampled creating a total of 35 rock profile samples. In addition, samples under five *T. erubescens* plants were also sub-sampled (total of eight samples). DNA was quantified using a spectrophotometer (Thermo Scientific Nanodrop 8000) and working solutions of 10ng/µl of DNA were made with sterilised water. DNA samples were amplified in a real-time thermal cycler (BioRad CFX96-C1000 touch) using four existing *T. erubescens* microsatellite primers (i.e. TE10, TE15, TE21, TE30; Anthony *et al.* 2016) and 20ng/µl of DNA each. Optimisation and testing of the priming conditions was completed, so the annealing temperature for the primers were 62°C, 53°C, 58°C and 53°C, respectively.

Figure 3.1.3a. Size of unidentified roots sampled from the rock profile that were assessed with microsatellite markers.

The thermal cycler recorded DNA amplification traces in real time and the results of sample amplification against three positive controls (or known references; DNA from leaf, shoot and root tissues of glasshouse grown *T. erubescens*) and two negative controls (amplification reaction without DNA and a blank sample, these were the negative controls) were analysed. Analysis involved scoring amplification quantities across the four primers. Criteria for assigning a sample as containing *T. erubescens* DNA, was a score of successful amplification in three or four of the loci. A positive score occurred when the amplification measured in the thermal cycler (CFU: c fluorescence unit) was at least 50% of the amplification quantity in the positive control (root tissue from the glasshouse) and amplification commenced before the amplification was initiated in the negative control (e.g. primer dimer irregularity; Ponchel *et al.* 2003).

DNA was extracted from all samples (average concentration of 568 ng/ul and a 1.7 ratio of A260/280). In summary, 9 unknown samples were considered to contain *T. erubescens* roots (30.9%) and all samples (eight) under *T. erubescens* plants were positive. The distribution of roots in the rock profile occurred from the rock crest (under plants) to the deepest sampling point at 400 cm (excluding samples at 80, 330 and 340 cm; (Figure 3.1.3b). This indicated that the plants could exploit the cracks, fissures and cavities of the rock strata to locate potential pockets of accessible moisture (see Elliott *et al.* 2018) for plant growth and survival. The presence of non-Tetratheca roots suggested that there is potential competition for these resources in the rock structure from other species. The rock profile had *T. erubescens* plants present on the crest and scattered down the profile (e.g. *T. erubescens* present halfway down and close to the base of the rock; Figure 3.1.3c), which means a limited interpretation of *T. erubescens* root length can be made, particularly as we could not trace roots through the profile from an individual plant.

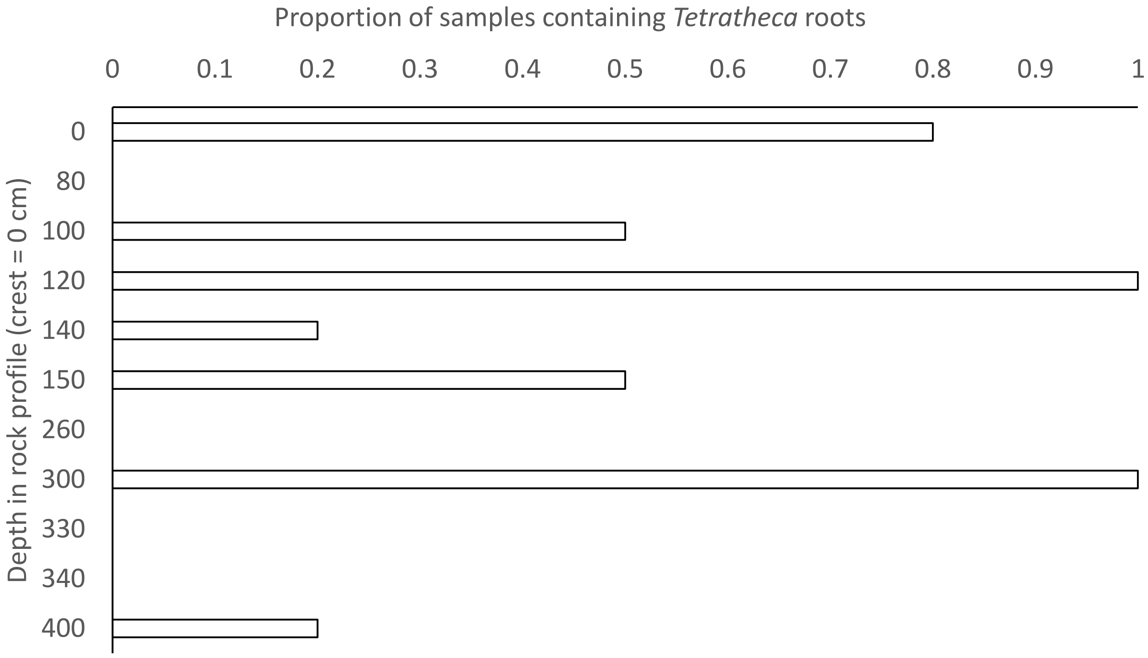


Figure 3.1.3b. Proportion of samples collected down through the rock profile (crest at 0cm to a depth of 400cm) that contained *T. erubescens* roots.

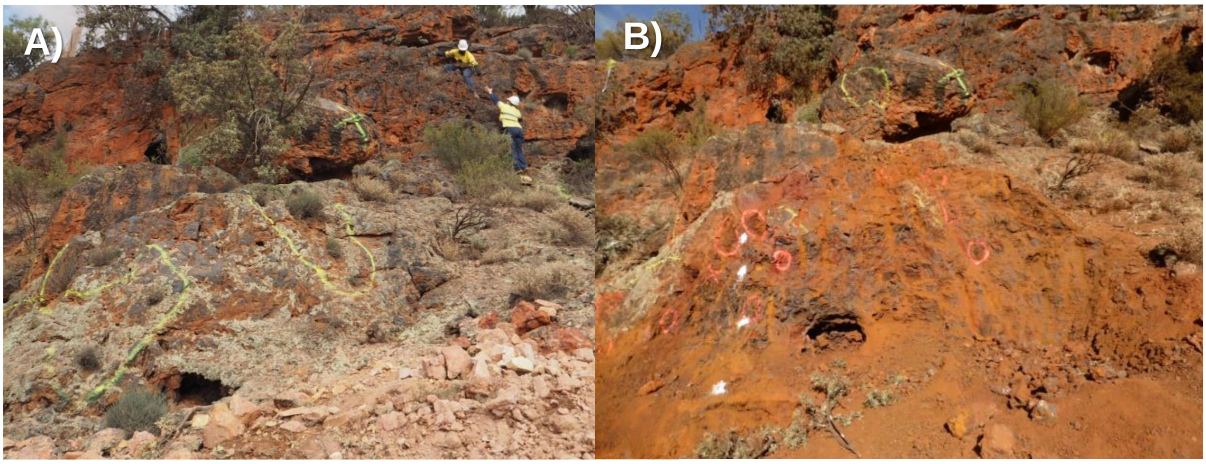


Figure 3.1.3c. Distribution of *T. erubescens* plants across the rock profile from the crest to the base of the rock structure. A) Before sampling: plants are indicated by yellow circles; and B) Former position of plants after sampling: plant locations are estimated by pink circles. White dots are the 1, 2, 3 and 4m distances down the profile from the crest. Photos: Jason Stevens.

3.1.4 Develop understanding of the environmental factors that underpin variation in plant function

Research outcomes:

- Within a 4-week window prior to ecophysiological measurements, the number of rainfall events (>4 events) and average rainfall (>2.5mm) were significantly associated with increasing stomatal conductance responses in both aspects.
- Plants on the SW-facing aspect were exposed to ca. 17% cooler (based on leaf temperatures) and ca. 95% wetter conditions, which were associated with increasing plant performance measures by at least 20-30%.
- Soil water potential and leaf temperatures are critical factors underpinning variation in plant function, with highest plant performance occurring in winter. Highest stress periods were associated with summer and autumn periods. Over the course of measurements, autumn represented the driest of all seasons.
- Critical thresholds reducing stomatal conductance to <50 mmol m^{-2} s^{-1} were modelled at 35°C for leaf temperatures, and -32 MPa for soil water potentials – these thresholds were commonly exceeded in the NE-facing aspect.

Background and methods

Spatiotemporal variation in plant performance across 13 seasons over three years were quantified, which provided the capacity to evaluate environmental factors underpinning variation in plant function. In summary, the results from Part 2: Section 3.1.2 demonstrate increased plant function during winter compared to summer, and differences between aspects. In order to further understand the factors associated with driving these differences, stomatal conductance was related to variables such as, rainfall (from climate data), high resolution soil microclimate data relating to soil moisture and temperature, and leaf temperatures across the north-eastern (NE; Plots 7 and 25) and south-western (SW; Plots 3 and 5) aspects.

To understand the effects of rainfall on plant function, the relationship between stomatal conductance, the cumulative rainfall, the average, and the count of rainfall events (by rainfall days) within the 4 weeks prior to measurements were analysed. All rainfall data from the nearest weather station from the Bureau of Meteorology were analysed (BOM: Koolyanobbing Station 012227). Measurements of stomatal conductance were analysed with aspects as a fixed effect, and seasons nested within years as the random error structure in the GLMM-analysis.

To understand the effects of soil water availability, volumetric soil moisture data were converted to soil water potential data using soil water retention curve relationships for the soil type of each logger station. After converting to soil water potentials, the relationships between stomatal conductance and soil water potentials were averaged for a 12 hour window prior to the stomatal conductance measurement, and mean soil water potential of the 4-week period prior to measurements.

To understand the effects of temperature, leaf temperature data were regressed against stomatal conductance data, which provided an organismal model. This relationship provided an insight into how plant performance was regulated intrinsically and the timepoint of the stomatal conductance measurement. Relationships of soil temperature were also investigated for a 12 hour window and a 4-week period prior to stomatal conductance measurements.

As data for soil/ leaf temperature and soil water potentials were captured across both aspects, measurements were blocked by seasons nested in years in the random error structure in the GLMM-analysis.

Results

Rainfall

In the 4-week window prior to ecophysiology measurements, rainfall in summer 2018 and 2021 were considered the wettest periods (cumulative rainfall of 67 and 80 mm, respectively) across all years. For these periods, rainfall was delivered across 13 and 6 days, with an average of 2.12 and 2.50 mm, for 2018 and 2021 summer seasons. The higher cumulative rainfall in 2021 was a result of fewer, but larger rainfall events compared to 2018 (Figure 3.1.4a). Autumn periods were characterised by lowest cumulative rainfall, frequency and averages in the 4-week window prior to measurements (Figure 3.1.4a). Winter rainfall in 2018 was delivered in a single rainfall event of 55 mm in the 4-week window prior to measurements, whilst rainfall in 2019 and 2020 were both characterised by seven events each, with an average of 0.3 and 1.15 mm, respectively. Spring was wettest in 2018, with 45 mm of accumulated rainfall delivered through 11 events, averaging 1.41 mm. In subsequent years, spring rainfall decreased in terms of accumulated rainfall, frequency and averages in the 4-week window prior to ecophysiology measurements.

The relationship between stomatal conductance and rainfall demonstrates strong partitioning between aspects, with SW-facing plots performing 20-30% higher compared to NE-facing plots (GLMM: $t = 10.58$, $P < 0.001$). The count/ number of rainfall events (≥4 rainfall events; GLMM: $t = 4.76$, $P = 0.001$) and average rainfall (≥2.5 mm; GLMM: $t = 2.03$, $P = 0.043$) in the 4-week window demonstrated significant increases (28-32%) in stomatal conductance (Figure 3.1.4a). In both of the aspects, cumulative rainfall did not have a strong effect on stomatal conductance (GLMM: $t = 0.65$, $P = 0.53$).

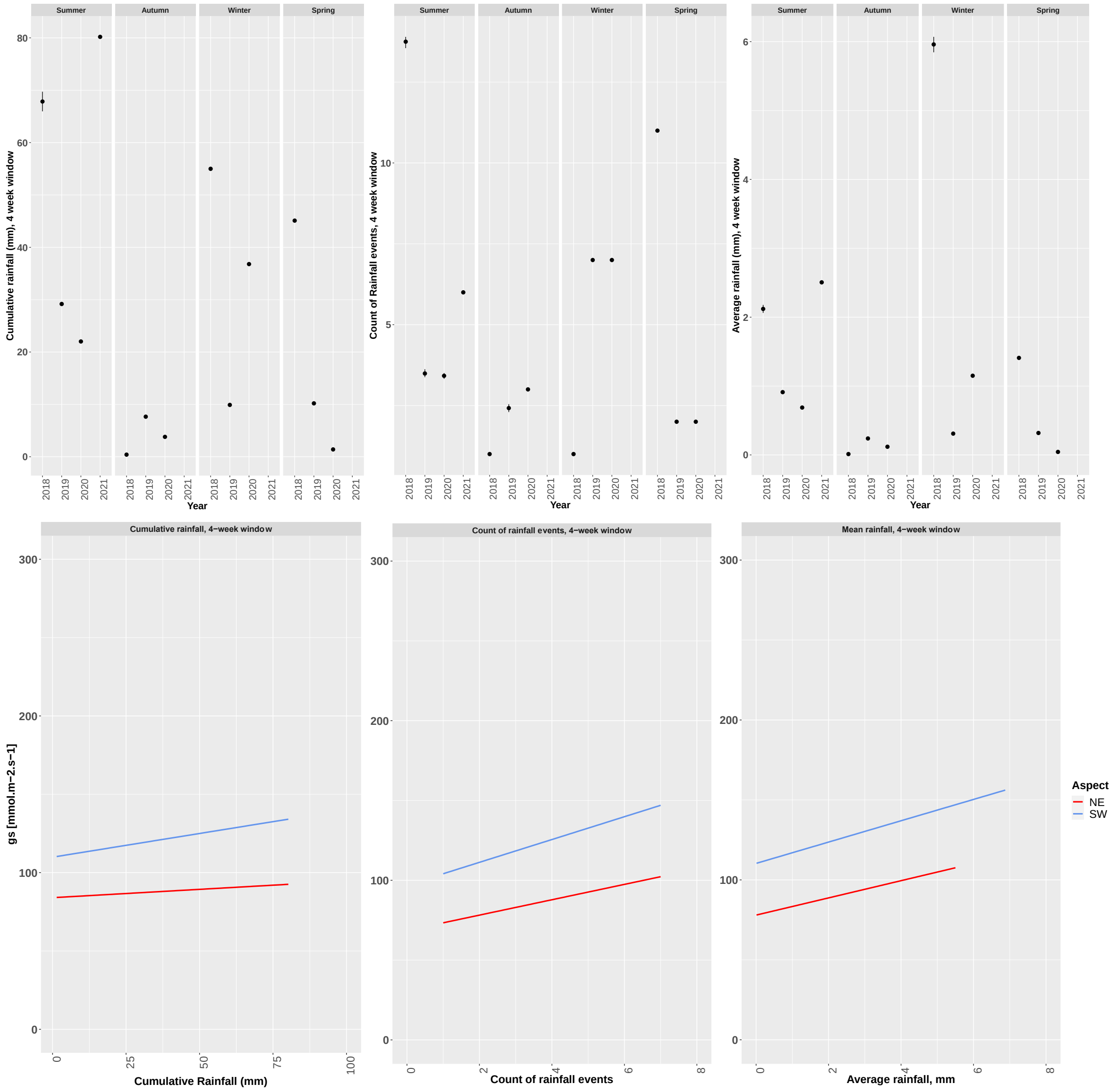


Figure 3.1.4a. Estimates (top panels) for cumulative rainfall, count of rainfall days and average rainfall are summarised for summer, autumn, winter and spring seasons between 2018 and 2020, with an additional measurement of summer in 2021. Relationships between stomatal conductance and rainfall (cumulative, count and average) in *Tetratheca erubescens* populations on north-eastern and south-western aspects (bottom panels). Measurements for rainfall were quantified over a 4-week period prior to measurements to characterise the environmental window of the season.

Soil water potentials

The soil water potential at the time of measurement was significantly associated with decreasing stomatal conductance responses in both aspects (GLMM: $t = 3.50$, $P < 0.001$), with population differences evident between aspects (GLMM: $t = 10.09$, $P < 0.001$). These differences were largely explained by the increased soil water potentials in the SW-facing aspect (average range: -0.36 MPa to -4.91 MPa) compared to the NE-facing aspect (average range: -5.27 MPa to -73.52 MPa) leading to 20-40% higher stomatal conductance responses. In both NE- and SW-facing aspects, the highest stomatal conductance responses (236-296 mmol m^{-2} s^{-1}) were generally associated with winter and spring soil water potentials, whilst the lowest responses (<100 mmol m^{-2} s^{-1}) were observed during autumn and summer (Figure 3.1.4b). Autumn and summer periods are also associated with soil water potentials dropping below -100 MPa, particularly in the NE-facing aspect. When considering these low soil water potentials, the critical threshold causing stomatal conductance responses to decline below 50 mmol m^{-2} s^{-1} was modelled at -32 MPa, suggesting the north-eastern aspect to be highly water stressed.

During the 4-week window prior to measurements (Figure 3.1.4b), the SW-facing plots were generally wetter (range: -0.22 MPa to -4.57 MPa) in contrast to the NE-facing plots (range: -12.95 MPa to -65.39 MPa), with declines in stomatal conductance by 48% ($GLMM_{SWPxSW}$: $t = 2.26$, $P = 0.024$) as the soil dried. For both aspects, wetter ranges were consistent with winter and spring seasons, whilst the drier ranges were associated with summer and autumn (Figure 3.1.4b). Populations in the north-eastern aspect were showing significantly on average 36% lower stomatal conductance responses, which was associated with the drier range in soil water potentials and a weak decline in stomatal conductance responses as soils dried (GLMM: $t = 0.97$, $P < 0.334$).

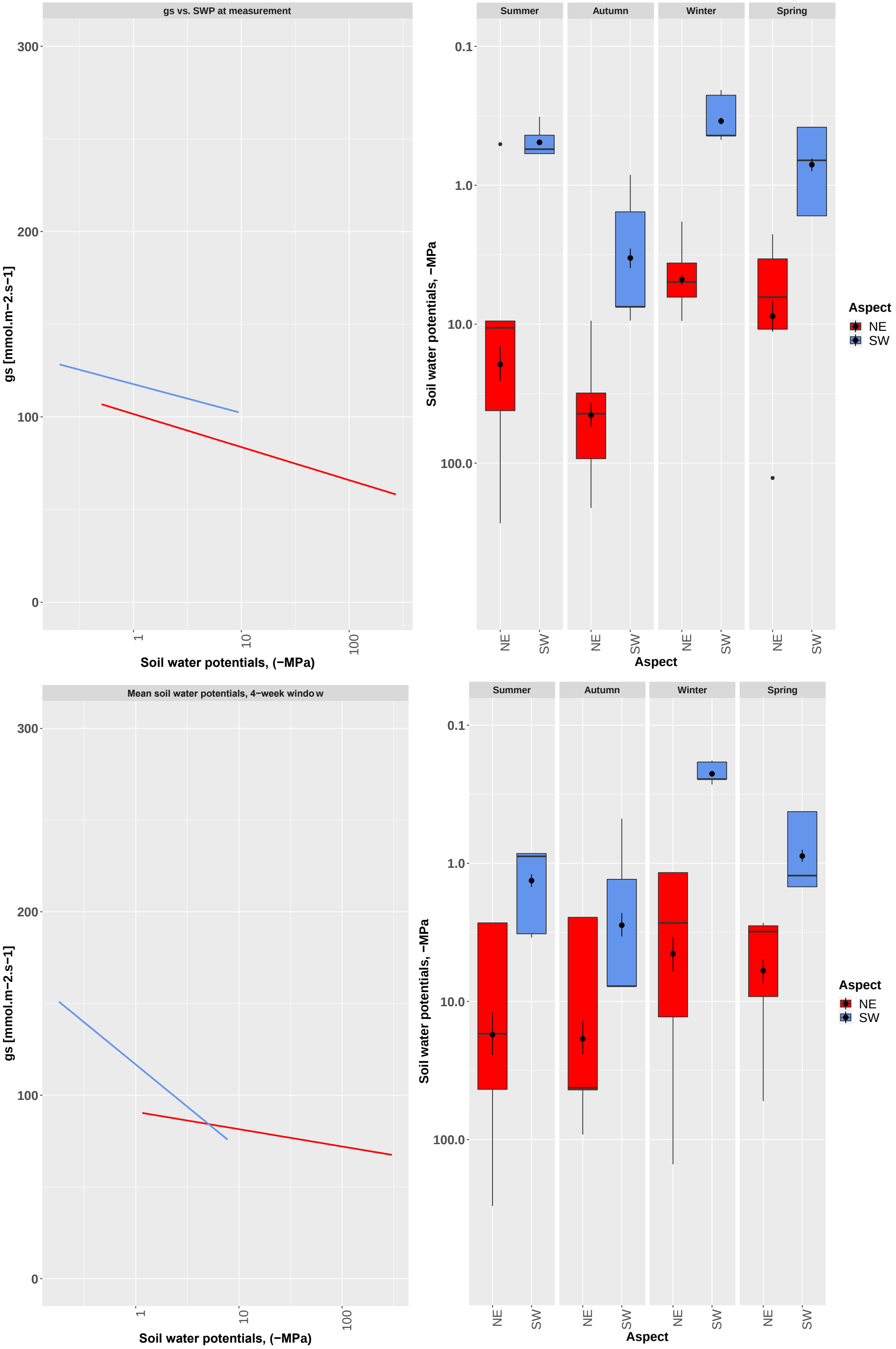


Figure 3.1.4b. Relationships between stomatal conductance and average soil water potential (left), and box and whisker plots (with mean point estimates and ± standard error) soil water potentials across seasons (right) in *Tetratheca erubescens* populations on north-eastern and south-western aspects. Soil water potential measurements were quantified at the time when stomatal conductance was measured (top panels) and over a 4-week period prior to measurements (bottom panels) to characterise the environmental window of the season.

Leaf/ soil temperature

A consistent trend between the leaf and soil temperatures are the differences between aspects, with the SW-facing plots appearing cooler (and wetter). The leaf temperature model also appears to describe the thermal relationships for stomatal conductance responses more appropriately than soil temperatures ($GLMM_{Tleaf}$ $R^2_c = 0.613$; $GLMM_{Tlsoilmeas}$ $R^2_c = 0.296$; $GLMM_{Tsoil4wk}$ $R^2_c = 0.324$).

Leaf temperatures were strong predictors for declines in stomatal conductance (GLMM: t = -14.77, $P < 0.001$), with differences between aspects (GLMM: t = 2.49, $P = 0.013$). The SW-facing plots demonstrated up to 22% higher responses compared to NE-facing plots at cooler leaf temperatures (<20°C). The increased responses largely coincided with winter seasons (Figure 3.1.4c), when rainfall raised soil moisture availabilities (Figure 3.1.4b). Despite comparatively cooler leaf temperatures in autumn, stomatal conductance responses during this period were lowered, due to decreased rainfall and lower soil water availability (Figure 3.1.4c).

Soil temperatures when stomatal conductance responses were measured and in the 4-week window prior to measurements were consistently cooler in the SW-facing aspect (Figure 3.1.4c), and during winter seasons. As with leaf temperatures, cooler soil temperatures during winter seasons were generally associated with higher stomatal conductance responses (Figure 3.1.4c), with increases in soil temperatures significantly decreasing stomatal conductance in both aspects ($GLMM_{Tmeas}$: t = -4.72, $P < 0.001$; $GLMM_{T4wk}$: t = -4.45, $P < 0.001$). There were higher soil temperatures (ca. 40°C) during the stomatal conductance measurements that coincided with the rainfall in the summer season in 2021 (Figure 3.1.4c), demonstrating the capacity of *T. erubescens* to respond to summer rainfall. However, the increased summer soil temperatures in the four weeks prior to measurements indicate lowest stomatal conductance responses regardless of aspect (Figure 3.1.4c). Further modelling the critical threshold for when stomata close on the leaf surface (e.g. 50 mmol m^{-2} s^{-1}), leaf temperatures were approximately 35°C in plants from both aspects, indicating a critical leaf temperature threshold for plant function. At leaf temperatures above 35°C plant performance can decrease by at least 3- to 4-fold from the maximum stomatal conductance response in both aspects (Figure 3.1.4c).

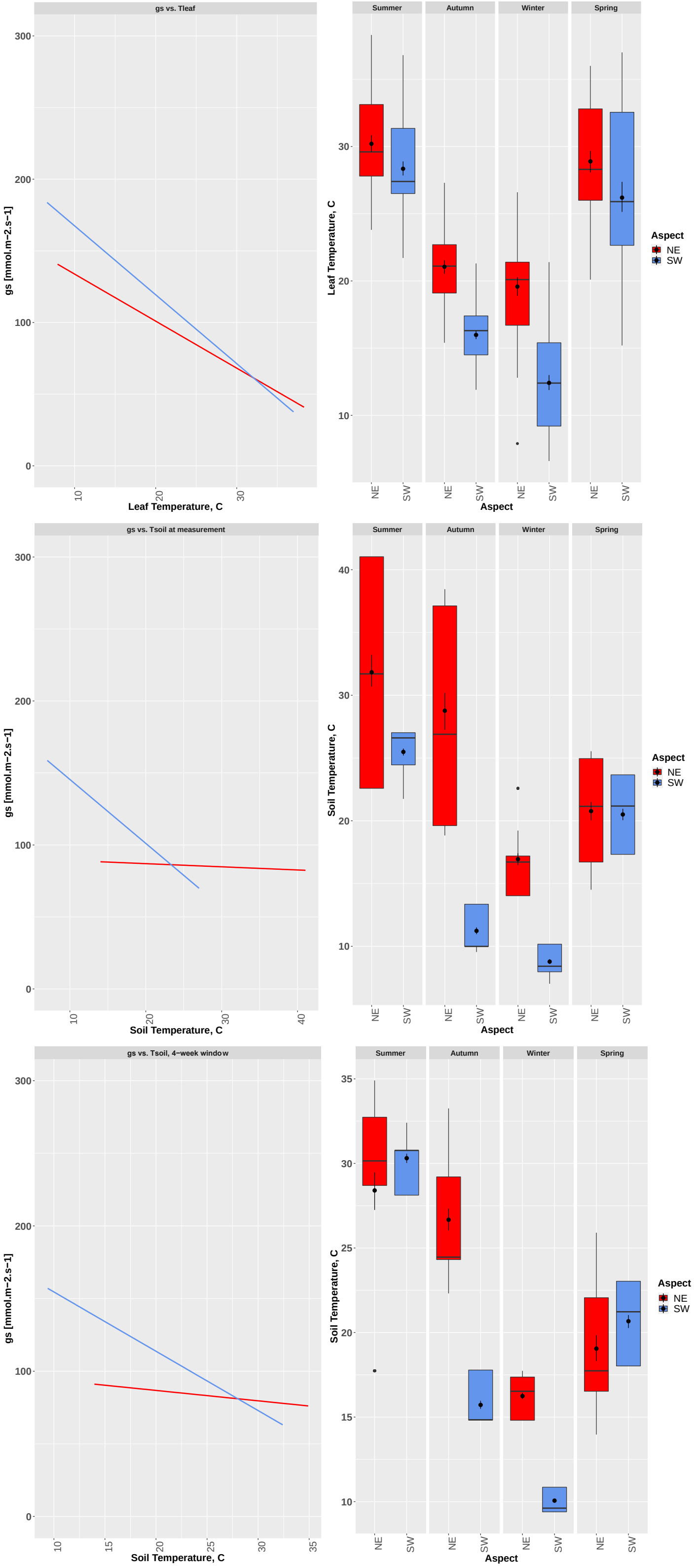


Figure 3.1.4c. Relationships between stomatal conductance and average temperature (left), and box and whisker plots (with mean point estimates and ± standard error) temperature across seasons (right) in *Tetratheca erubescens* populations on north-eastern and south-western aspects. Leaf temperatures (top panels) were quantified at the time of stomatal conductance measurement only, while soil temperatures were quantified at the time when stomatal conductance were measured (middle panels) and over a 4-week period prior to measurements (bottom panels) to characterise the environmental window of the season.

3.1.5 Compare plant function (chlorophyll fluorometry, leaf gas exchange, and plant water status) of plants growing in natural and translocated sites

Research outcomes:

- Ecophysiological assessments were conducted on cuttings that were planted in 2017-2020 translocation trials with the aim to compare plant function from establishing plants with plants in natural sites. Measurements were quantified using the same approach as outlined in Section 3.1.2, by quantifying stomatal conductance, leaf temperatures and chlorophyll fluorescence. As there was a strong difference between the aspects in natural sites for ecophysiological functioning measurements were conducted in translocation sites T23 and T6 and compared against natural/ reference sites on the same aspect.
- Plants in natural reference sites demonstrated increased stomatal conductance (see Elliott *et al.* 2021, Figure 3.1.5a) and lower leaf temperatures (see Elliott *et al.* 2021, Figure 3.1.5b) at the commencement of measurements compared to the cuttings in translocation sites. In 2019, older and more established cuttings generally demonstrated higher ecophysiological functioning (e.g. 2017 and 2018; increased stomatal conductance and chlorophyll fluorescence) compared to cuttings from the 2019 translocation trial. The increased ecophysiological functioning was likely explained by the cuttings having established and survived in their planting locations, and their roots likely accessing moisture resources in the rock profile.
- The cuttings from the 2019 translocation were also exposed to increased environmental stress after planting, due to the low rainfall in 2019, which resulted in highly reduced ecophysiological functioning (e.g. stomatal conductance < 50 $mmol.m^{-2}.s^{-1}$; Fv/Fm < 0.1; see Elliott *et al.* 2021, Figures 3.1.5a,b) and increased mortality rates in spring 2019 and summer 2019/20.
- There were elevated performance values from the 2017 cuttings in the SW-facing aspect compared to natural plants and 2018 cuttings in the summer of 2020. These measurements coincided with rainfall events of >20 mm that are believed to have saturated the drill holes into which the cuttings were planted. The performance in the summer 2020 for cuttings in the NE-facing aspect was generally either same for natural plants and cuttings from the 2017 translocation, or reduced for cuttings from the 2018 translocation year. The lower performance could be attributed to the plants being of smaller size or still establishing in their niche compared to the 2017 cuttings.
- From the 2018 and 2020 translocations, ecophysiological function decreased strongly in NE-aspects from winter into spring and were like the performance from naturally occurring plants, while there was reduced/ conservative performance from the 2017 cuttings between the seasons (see Elliott *et al.* 2021, Figure 3.1.5a). In the SW-facing aspect, all cuttings were performing lower compared to natural plants in winter, while in summer there was no difference between natural plants and older planting years (e.g. 2017 and 2018). The cuttings that were recently planted in the winter of 2020 all demonstrated a strong reduction in ecophysiological function, with lowest performance measured in the 2021 summer period.
- The leaf temperatures were relatively similar between natural/ reference sites and translocated plants, except for measurements occurring in the winter, 2020 season

where all translocated cuttings in the NE-facing aspect were demonstrated elevated leaf temperatures compared to natural plants (see Elliott *et al*. 2021, Figure 3.1.5b). The recent summer in 2021 demonstrated the highest leaf temperatures.

- This research demonstrates that winter consistently represents the period of highest plant health across all sites, while spring and summer represent the periods of highest stress (see Elliott *et al*. 2021, Figure 3.1.5c). This is supported by elevated leaf temperatures and lower chlorophyll fluorescence during this period. For 2019 and 2020 plantings, reduced ecophysiological function also coincided with increased mortality rates during this period.
- It is recommended that intervention measures should be undertaken prior to the onset of the first summer period to increase plant survival – as we have demonstrated that water stress and temperature decrease physiological function - management strategies should consider irrigating sites to increase water availability, and shading plants to decrease temperature of plants.

3.3 *Soil biological function in natural and translocation sites.*

3.3.1 Assess biological communities of soils where *T. erubescens* grow

Research outcomes:

- The biological communities of soils consist of bacteria, fungi, viruses, archea and protists, which can influence plant survival and function because of mutualistic-beneficial relationships with these microbes. These relationships may afford plants access to nutrients or drought and pathogen resistance. Understanding species diversity and composition of the soil microbe community can identify if this community is unique to the root zone area of *T. erubescens* and may suggest specific relationships that need investigation, as this may define soil communities to improve translocation success.
- Genomic analysis of soil samples suggests that there were distinct bacterial communities under *T. erubescens* plants in comparison to locations that were not associated with *in situ* plants. However, this is also accompanied by a lot of variation among the samples within this group, which is similar to the amount of variation among samples that were not associated with *in situ* plants.
- Genomic analysis of soil samples suggests that there were distinct fungal communities under *T. erubescens* plants in comparison to two of the four other habitats that were not associated with *in situ* plants.
- Whilst recognising distinct bacterial and fungal communities in the soils associated with *T. erubescens*, it is not clear of the functional significance of this result. For example, further research would be required to understand if *T. erubescens* is dependent on this soil microbial community (or certain species) for growth, and whether soils at potential translocation sites require this microbial community to support *T. erubescens*.

Soils were sampled in July 2017 and October 2017 from six locations (Figure 3.3.1a) and sent to the Australian Genome Research Facility (AGRF, Adelaide; Plant Genomics Centre at the University of Adelaide) for genome sequencing. These 31 soil samples were from several habitat types: the root zone area of *T. erubescens* plants in cliff and top of ridge habitat; adjacent to *T. erubescens* plants in cliff habitat (>1m); random locations in cliff, top of ridge or slope habitat at Koolyanobbing Range (Table 3.3.1a).

Samples were extracted for microbial DNA, PCR-amplified with bacterial (16S) and fungal (ITS) markers and sequenced on the Illumina MiSeq platform. Sequences were compared to a global genebank database (GreenGenes; greengenes.secondgenome.com) to determine what species, or operational taxonomic units (OTUs) if species could not be identified, were in the samples and their relative abundance based on the sequencing information.

Table 3.3.1a. Description of the soil samples used for genomic sequencing. Their locations are indicated on the map below (Figure 3.3.1a). The codes for each sample that are shown in Figures 3.3.1c and 3.3.1d are also included.

Site	Sample type	Habitat	No. samples	Sample codes
S1	Root zone of *Tetratheca* plants	Cliff	4	S1_TERB S1_TE21 S1_TE23 S1_TE24
	Adjacent to *Tetratheca* plants	Cliff	5	S1_NTE_25 S1_NTE_23 S1_NTE_N S1_NTE_S S1_NTE_24
	Random	Cliff	5	S1_R1 S1_R2 S1_R3 S1_R4 S1_R5
S2	Random	Slope	1	S2_R1
S3	Random	Slope	1	S3_R1
S4	Random	Cliff	1	S4_R1
S5	Random	Top of ridge	5	S5_R1 S5_R2 S5_R3 S5_R4 S5_R5
S6	Root zone of *Tetratheca* plants	Top of ridge	9	S6_TE1 S6_TE2 S6_TE3 S6_TE4 S6_TE5 S6_TE8 S6_TE16 S6_TE17 S6_TE20

Microbial community richness and composition

Bacteria

The average number of bacteria species (or operational taxonomic units; OTUs) was similar among all habitats, except the random locations on top of the ridge which had the highest species richness (Figure 3.3.1b.a). In terms of species composition, the relative abundance of bacteria OTUs was consistent among the habitat types, with an average of 80.5% of OTUs occurring at very low frequencies (OTUs with < 0.05% relative abundance in a sample). Interestingly, samples from under *T. erubescens* plants contained OTUs that occurred at higher frequencies (OTUs with 15-20% relative abundance in a sample), whereas the other samples only had comparatively lower frequencies of occurrence (OTUs with < 15% relative abundance in a sample). This indicates that soil bacterial communities are likely dominated by only a few OTUs in all samples, but in samples from under *T. erubescens* plants these dominant OTUs occur at a higher relative abundance than the dominant OTUs in the other samples. Figure 3.3.1c.a illustrates the complexity of soil bacterial communities detected in all the soil samples analysed, regardless of their location.

Fungi

The average number of fungi species (or operational taxonomic units; OTUs) was mixed among habitat types, with the samples adjacent to *T. erubescens* plants and those from random locations on top of the ridge having the highest OTUs richness (Figure 3.3.1b.b). In terms of species composition, the relative abundance of fungi OTUs was similar between samples from under *T. erubescens* plants and random locations, with an average of 88% of OTUs occurring at very low frequencies (OTUs with < 0.05% relative abundance in a sample). Whereas samples from adjacent to *T. erubescens* plants having an average of 74.5% of OTUs occurring at very low frequencies. Unlike the bacterial community, samples from all habitat types contained OTUs that occurred at higher frequencies (OTUs with 15-20% relative abundance in a sample). This indicates that fungi communities are all likely dominated by only a few OTUs and these dominant OTUs occur at a higher relative abundance. Figure 3.3.1c.b illustrates the complexity of soil fungal communities detected in all the soil samples analysed, regardless of their location.

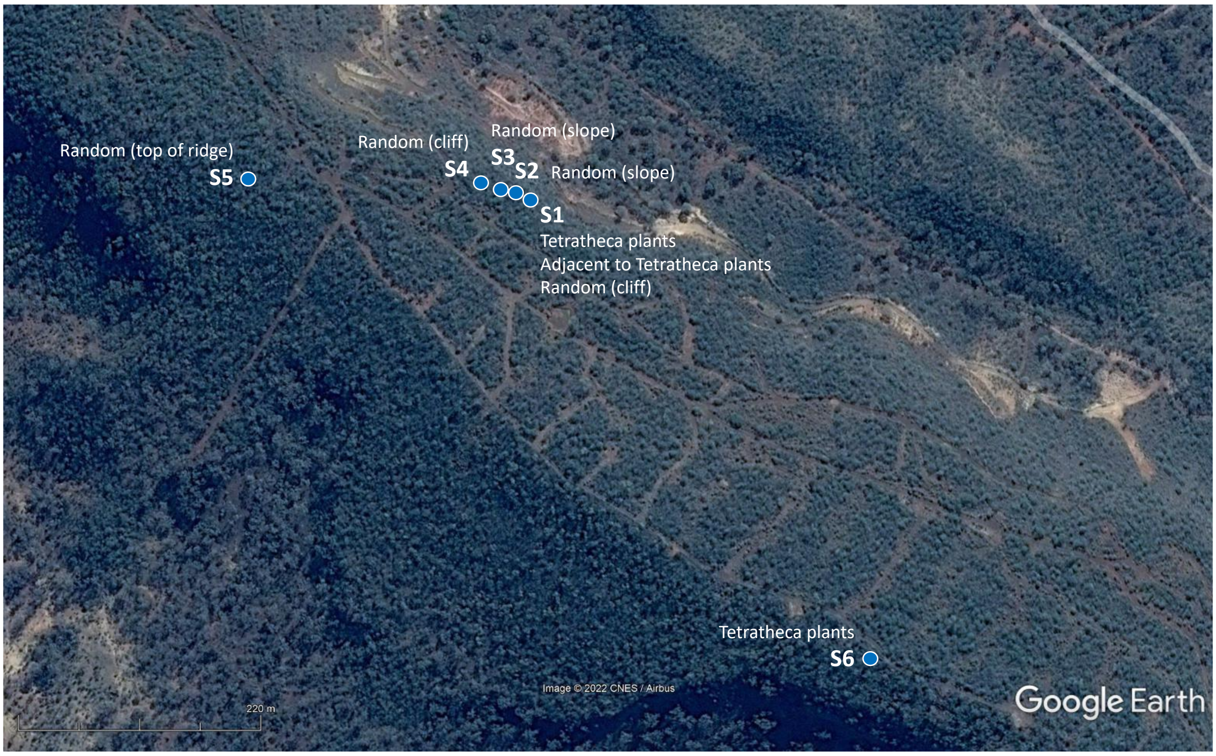


Figure 3.3.1a. Soil sample locations that were sampled at Koolyanobbing Range. Location S1 sampled habitat types of the root zone area of *T. erubescens* plants in cliff (*n* = 4); adjacent to *T. erubescens* plants in cliff habitat (>1m; *n* = 5 samples); and random locations in cliff (*n* = 5). Locations S2 and S3 each sampled a random location on the slope (*n* = 2). Location S4 sampled a random location in cliff habitat (*n* = 1). Location S5 sampled random locations in top of ridge habitat (*n* = 5). Location S6 sampled the root zone area of *T. erubescens* plants in top of ridge habitat (*n* = 9).

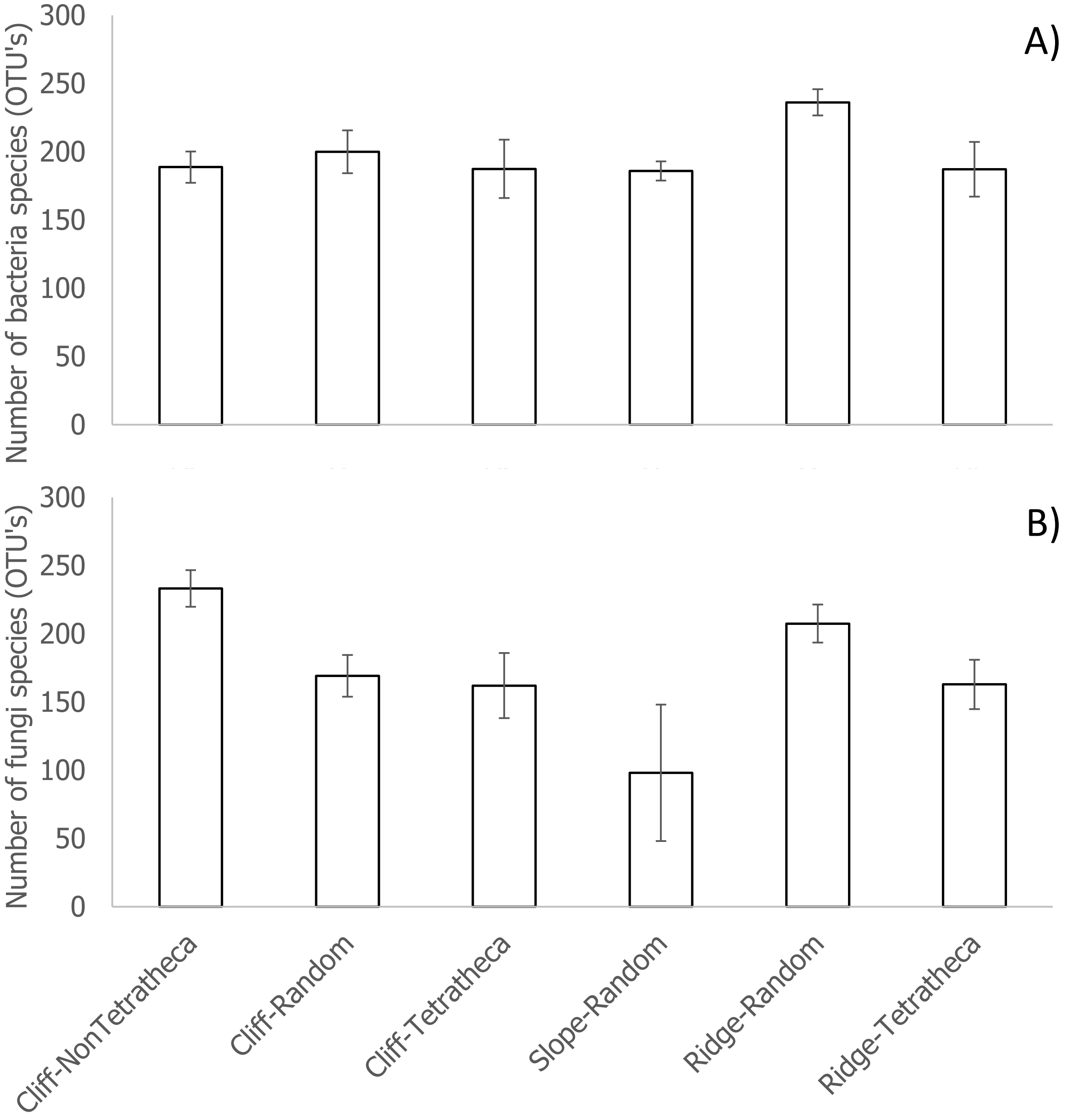


Figure 3.3.1b. Average species (or operational taxonomic units; OTUs) richness of A) bacteria and B) fungi communities at the six different habitats that were sampled at Koolyanobbing Range (mean ± standard error). Cliff-NonTetratheca: adjacent to *T. erubescens* plants in cliff habitat (>1m; *n* = 5 samples); Cliff-Random: random locations in cliff (*n* = 6); Cliff-Tetratheca: root zone area of *T. erubescens* plants in cliff (*n* = 4); Slope-Random: random locations on the slope (*n* = 2); Ridge-Random: random locations on top of ridge (*n* = 5); and Ridge-Tetratheca: root zone area of *T. erubescens* plants on top of the ridge (*n* = 9).

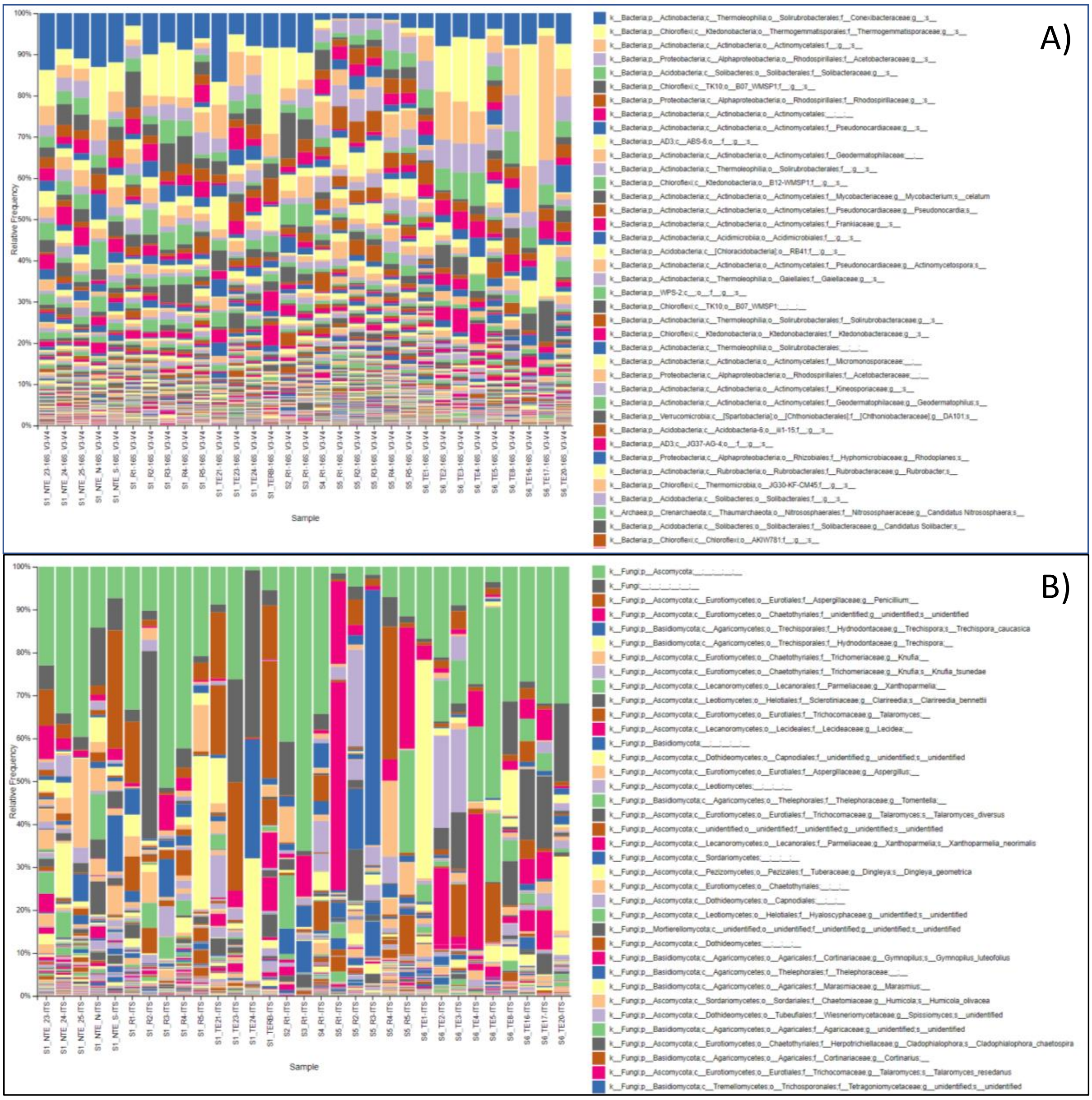


Figure 3.3.1c. Species (or operational taxonomic units; OTUs) diversity and composition of A) bacteria and B) fungi communities at the six different habitats that were sampled at Koolyanobbing Range (see Table 3.3.1a for site description).

Microbial community uniqueness

Bacteria

Figure 3.3.1d.a shows the relative similarity of soil bacterial communities among each sample and shows that there were distinct bacterial communities under *T. erubescens* plants in comparison to the other habitat types. This is illustrated by samples of this habitat type (light blue triangles) not overlapping in space with any other habitat types (i.e. are dissimilar) and tend to be grouped together in the lower half of the figure (i.e. are more similar to each other). Pairwise comparisons (statistical test: multi-response permutation procedure performed) among the habitat types showed that bacterial communities from the root zone area of *T. erubescens* plants were significantly different to any other habitat type ($P < 0.05$).

Fungi

Figure 3.3.1d.b shows the relative similarity of soil fungal communities among each sample was weaker than that observed in the bacterial communities and suggests that there was greater similarity of fungal communities under *T. erubescens* plants in comparison to the other habitat types. This is illustrated by samples of this habitat type (light blue triangles) overlapping in space with the other habitat types (i.e. are similar). However, pairwise comparisons (statistical test: multi-response permutation procedure performed) among the habitat types showed that even in this weaker community structure, the fungal communities from the root zone area of *T. erubescens* plants were significantly different to any other habitat type ($P < 0.05$), except for samples from the random ridge or random cliff habitats ($P > 0.05$).

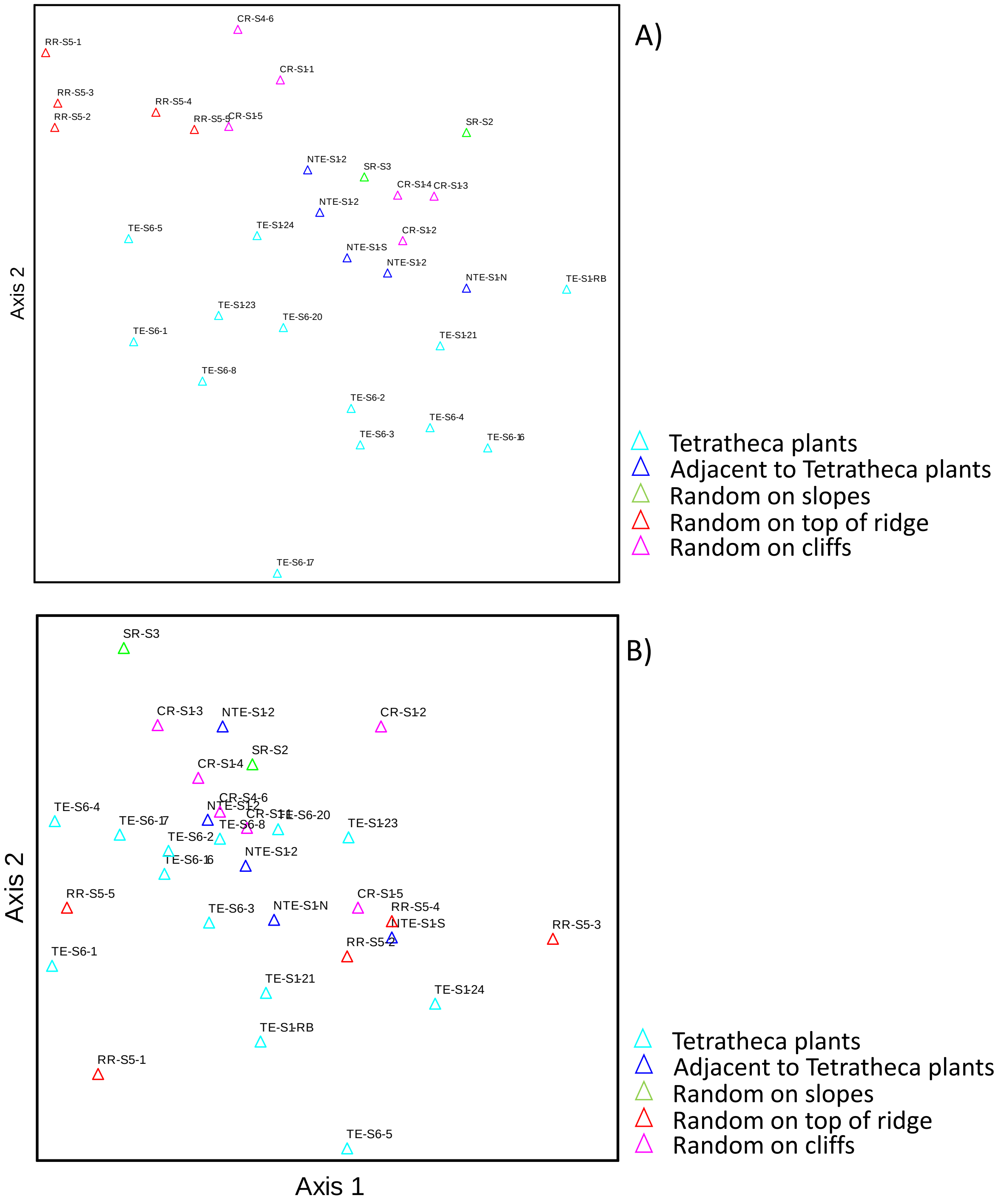


Figure 3.3.1d. Non-metric multi-dimensional scaling (nMDS) analysis of the relative similarity in species (or operational taxonomic units; OTUs) of A) bacteria and B) fungi communities in the five habitat types (pooled samples from under *T. erubescens* plants on cliff and ridge habitat) that were sampled at Koolyanobbing Range (see Table 3.3.1a for site description).

PROGRAM SCHEDULE

The five-year project is complete (Table 4). Each program had specific trajectories and the schedule implementation reflected this focus on *ex situ* research, initial *in situ* conditions and on developing *in situ* conditions in translocated populations.

Table 4 Program schedule - completed.

	2017/18				**2018/19**				**2019/20**				**2020/21**				**2021/22**			
	W	**S**	**S**	**A**	**W**	**S**	**S**	**A**	**W**	**S**	**S**	**A**	**W**	**S**	**S**	**A**	**W**	**S**	**S**	**A**
Pr.1 Seed biology																				
1.1 Dormancy and germination																				
test 'best bet' dormancy mode & germination	▨																			
Refine tests: wet/dry cycling			▨	▨	▨															
temperature and water potential tests						▨	▨													
1.2 Seed enhancement																				
Priming			▨																	
develop pellet design				▨																
preliminary pelleting field trials					▨	▨														
refine pellet design							▨	▨												
test pelleting in field									▨	▨										
Pr.2 Translocation and monitoring																				
2.1 Optimising translocation approaches																				
establish initial translocation sites	▨																			
collect seed and propagate material for Y2		▨	▨																	
develop sites for Y2 trial			▨	▨																
2nd year translocation					▨															
preliminary pelleting field trials					▨	▨														
3rd year translocation: test pelleting in field									▨	▨										
4th year translocation													▨	▨						
Monitor translocation		▨				▨				▨				▨				▨		
2.2 Survival, growth and reproduction																				
Demographic survey		▨				▨				▨				▨				▨		
demographic modelling																			▨	
Pr.3 Plant function, habitat and substrate interactions																				
3.1 Plant function, condition and water use																				
regular plant function monitoring: in natural systems			▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	
in translocation											▨	▨	▨	▨	▨	▨	▨	▨	▨	
3.2 Soil - nutrient acquisition interactions																				
collect and analyse soils: in natural sites		▨	▨																	
in translocation														▨	▨					
3.3 Soil biological function																				
collect materials and undertake molecular analysis: in natural system	▨																			
in translocation																	▨			
mycorrhizal assessment						▨	▨													

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APPENDIX 1

Items for Stage 1 Offset Plan

Table S1.1. Items from the Stage 1 *Tetratheca erubescens* Offsets Plan August 2017, Cliffs Asia Pacific Iron Ore.

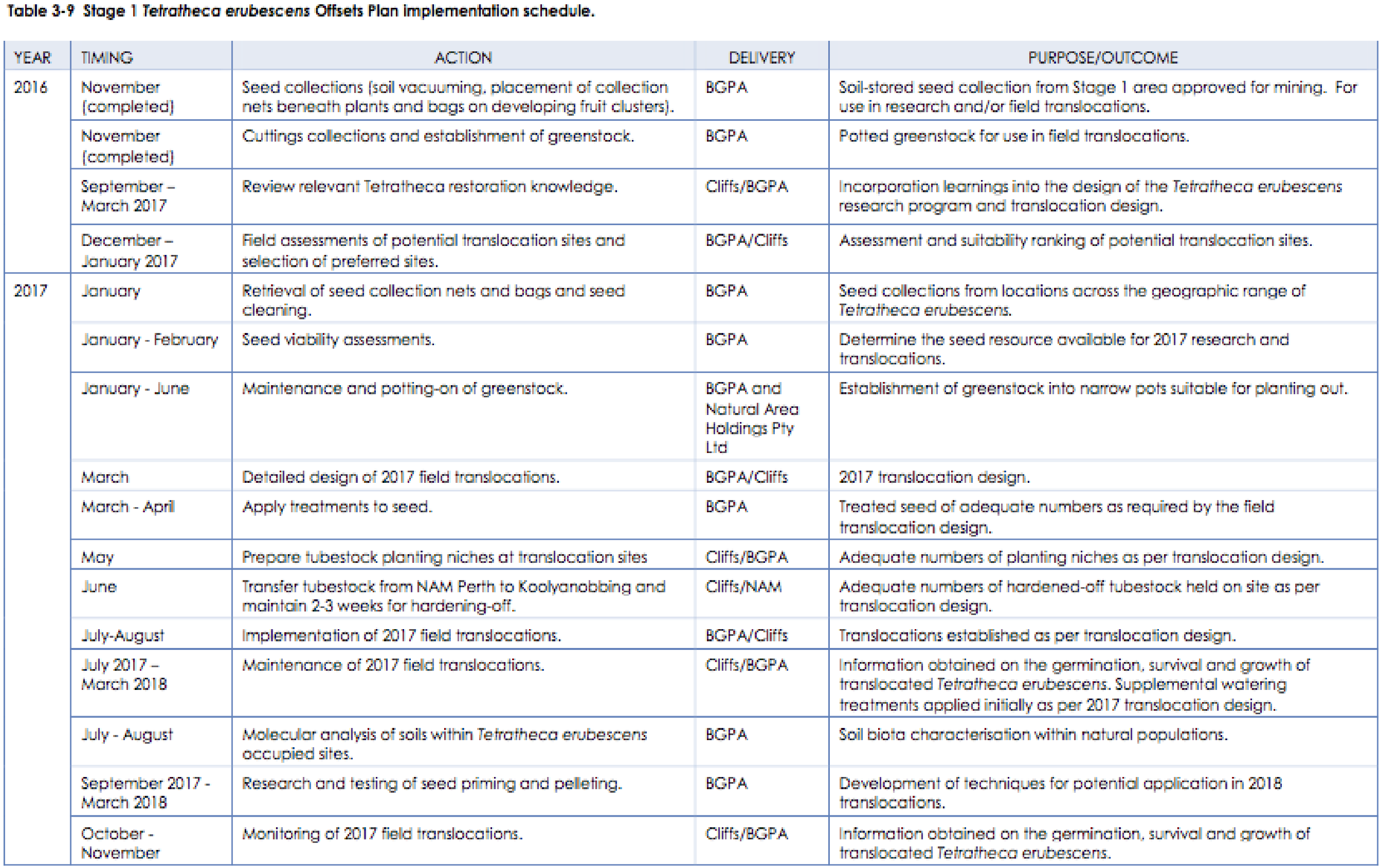
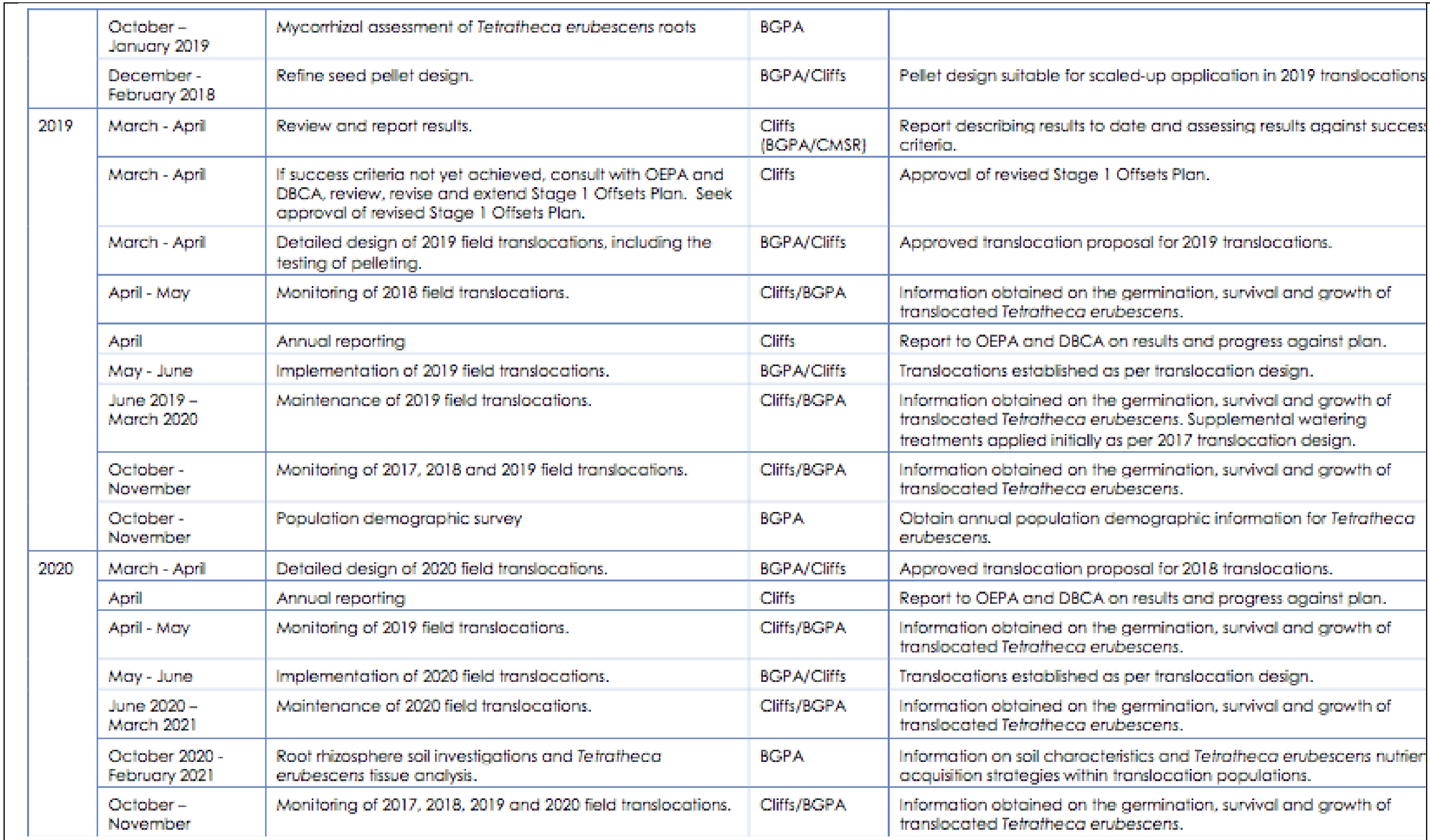


Table 3-9 Stage 1 *Tetratheca erubescens* Offsets Plan implementation schedule.

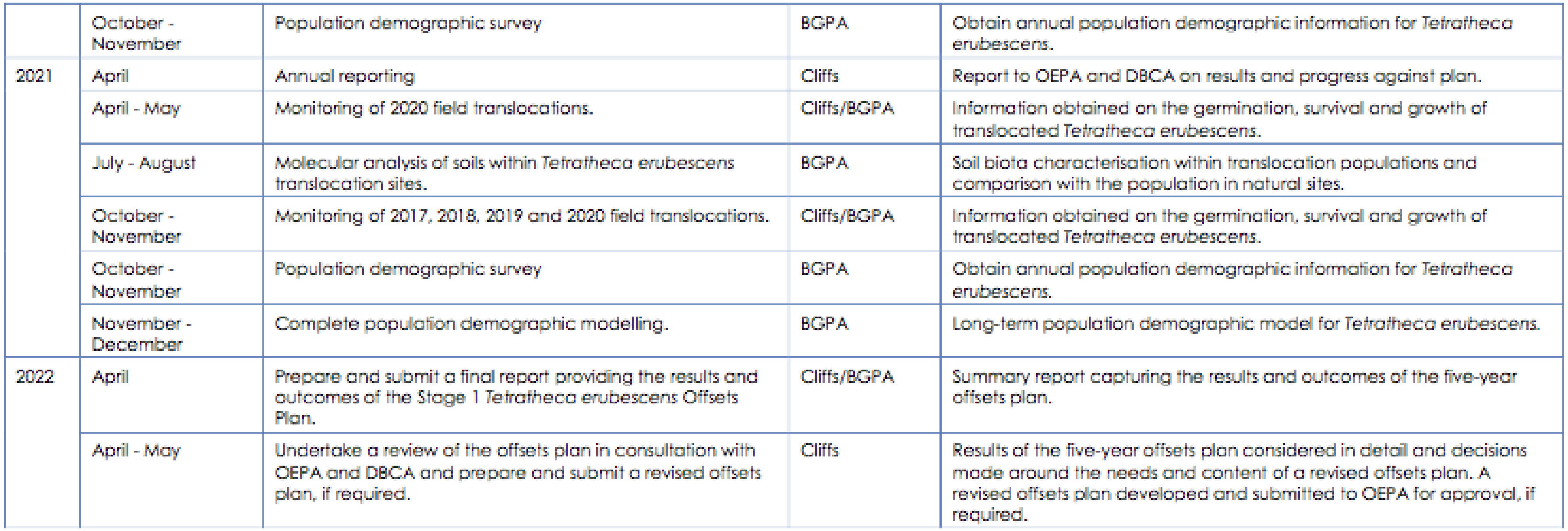
YEAR	TIMING	ACTION	DELIVERY	PURPOSE/OUTCOME		
2016	November (completed)	Seed collections (soil vacuuming, placement of collection nets beneath plants and bags on developing fruit clusters).	BGPA	Soil-stored seed collection from Stage 1 area approved for mining. For use in research and/or field translocations.		
	November (completed)	Cuttings collections and establishment of greenstock.	BGPA	Potted greenstock for use in field translocations.		
	September – March 2017	Review relevant Tetratheca restoration knowledge.	Cliffs/BGPA	Incorporation learnings into the design of the *Tetratheca erubescens* research program and translocation design.	Completed (2017 report)	
	December – January 2017	Field assessments of potential translocation sites and selection of preferred sites.	BGPA/Cliffs	Assessment and suitability ranking of potential translocation sites.	Completed (2017 report)	
2017	January	Retrieval of seed collection nets and bags and seed cleaning.	BGPA	Seed collections from locations across the geographic range of *Tetratheca erubescens*.	Completed (2017 report)	
	January - February	Seed viability assessments.	BGPA	Determine the seed resource available for 2017 research and translocations.	Completed (2017 report)	
	January - June	Maintenance and potting-on of greenstock.	BGPA and Natural Area Holdings Pty Ltd	Establishment of greenstock into narrow pots suitable for planting out.	Completed (2017 report)	
	March	Detailed design of 2017 field translocations.	BGPA/Cliffs	2017 translocation design.	Completed	
	March - April	Apply treatments to seed.	BGPA	Treated seed of adequate numbers as required by the field translocation design.	Completed (2017 report)	
	May	Prepare tubestock planting niches at translocation sites	Cliffs/BGPA	Adequate numbers of planting niches as per translocation design.	Completed (2017 report)	
	June	Transfer tubestock from NAM Perth to Koolyanobbing and maintain 2-3 weeks for hardening-off.	Cliffs/NAM	Adequate numbers of hardened-off tubestock held on site as per translocation design.		
	July-August	Implementation of 2017 field translocations.	BGPA/Cliffs	Translocations established as per translocation design.	Completed (2018 report)	
	July 2017 – March 2018	Maintenance of 2017 field translocations.	Cliffs/BGPA	Information obtained on the germination, survival and growth of translocated *Tetratheca erubescens*. Supplemental watering treatments applied initially as per 2017 translocation design.	Completed (2018 report)	
	July - August	Molecular analysis of soils within *Tetratheca erubescens* occupied sites.	BGPA	Soil biota characterisation within natural populations.	Completed (2022 report)	P2 Section 3 pp. 85-91
	September 2017 - March 2018	Research and testing of seed priming and pelleting.	BGPA	Development of techniques for potential application in 2018 translocations.	Complete (2017 report)	
	October - November	Monitoring of 2017 field translocations.	Cliffs/BGPA	Information obtained on the germination, survival and growth of translocated *Tetratheca erubescens*.	Completed (2018 report)	

	October - November	Population demographic survey	BGPA	Obtain annual population demographic information for *Tetrathec* *erubescens*
	October 2017 - January 2018	Seed and cuttings collections.	BGPA	Obtain adequate viable seed and cuttings for 2018 research and translocations.
	October 2017 - February 2018	Root rhizosphere soil investigations and *Tetratheca erubescens* tissue analysis.	BGPA	Information on soil characteristics and *Tetratheca erubescens* nutri acquisition strategies within natural populations.
	December 2017 - December 2021	Monitoring of plant physiology and function in natural and restored populations	BGPA/Cliffs	Build a profile of physiological functioning across life stages of *Tetratheca erubescens* and compare with translocated individual
2018	January 2018 – May 2019	Development and testing of seed priming, coating and pelleting methods.	BGPA/Cliffs	Determine suitable methods and complete laboratory/greenhous testing.
	February	Review and report results.	Cliffs/BGPA	Report describing results to date and assessing results against succ criteria.
	February-March	Consult with technical specialists, OEPA and DBCA regarding results and 2018 research and translocation plans.	Cliffs	Recommendations regarding 2018 program.
	February - March	Evaluate potential translocation sites for 2018 translocations.	BGPA/Cliffs	Preferred translocation sites for 2018.
	February - May	Follow-up research and testing of methods to refine direct seeding and/or greenstock translocation methods.	BGPA/Cliffs	Refined translocation methods available for application in 2018.
	March - April	Detailed design of 2018 field translocations.	BGPA/Cliffs	Approved translocation proposal for 2018 translocations.
	April-May	Monitoring of 2017 field translocations.	Cliffs/BGPA	Information obtained on the germination, survival and growth of translocated *Tetratheca erubescens*.
	April	Annual reporting	Cliffs	Report to OEPA and DBCA on results and progress against plan.
	May - June	Implementation of 2018 field translocations.	BGPA/Cliffs	Translocations established as per translocation design.
	June 2018 – March 2019	Maintenance of 2018 field translocations.	Cliffs/BGPA	Information obtained on the germination, survival and growth of translocated *Tetratheca erubescens*. Supplemental watering treatments applied initially as per 2017 translocation design.
	June - November	Preliminary field testing of pelleting methods.	BGPA	Determine if pelleting is likely to be suitable for field applications.
	October - November	Monitoring of 2017 and 2018 field translocations.	Cliffs/BGPA	Information obtained on the germination, survival and growth of translocated *Tetratheca erubescens*.
	October - November	Population demographic survey	BGPA	Obtain annual population demographic information for *Tetrathec* *erubescens*
	October – January 2019	Seed temperature and water potential testing.	BGPA	Information to refine optimal seed pre-treatments.
	October – January 2019	Seed and cuttings collections.	BGPA	Obtain seed and cuttings for 2019 translocations.

Completed (2018 report) Completed (2018 report) Completed (2020 report) Completed (2022 report) Completed (2021 report) Complete Complete Complete (2019 report) Complete Complete (2019 report) Complete (2019 report) Part complete (2019 report) Complete Complete (2019 report) Complete (2019 report) Complete (2020 report) Complete (2020 report)	P2 Section 3 pp. 83-84



	October – January 2019	Mycorrhizal assessment of *Tetratheca erubescens* roots	BGPA		Complete (2022 report)	P1 Section 3 pp. 27-28
	December - February 2018	Refine seed pellet design.	BGPA/Cliffs	Pellet design suitable for scaled-up application in 2019 translocations	Completed (2021 report)	
2019	March - April	Review and report results.	Cliffs (BGPA/CMSR)	Report describing results to date and assessing results against succes criteria.	Complete	
	March - April	If success criteria not yet achieved, consult with OEPA and DBCA, review, revise and extend Stage 1 Offsets Plan. Seek approval of revised Stage 1 Offsets Plan.	Cliffs	Approval of revised Stage 1 Offsets Plan.		
	March - April	Detailed design of 2019 field translocations, including the testing of pelleting.	BGPA/Cliffs	Approved translocation proposal for 2019 translocations.	Complete	
	April - May	Monitoring of 2018 field translocations.	Cliffs/BGPA	Information obtained on the germination, survival and growth of translocated *Tetratheca erubescens*.	Complete (2020 report)	
	April	Annual reporting	Cliffs	Report to OEPA and DBCA on results and progress against plan.		
	May - June	Implementation of 2019 field translocations.	BGPA/Cliffs	Translocations established as per translocation design.	Complete (2020 report)	
	June 2019 – March 2020	Maintenance of 2019 field translocations.	Cliffs/BGPA	Information obtained on the germination, survival and growth of translocated *Tetratheca erubescens*. Supplemental watering treatments applied initially as per 2017 translocation design.	Complete (part) (2020 report)	
	October - November	Monitoring of 2017, 2018 and 2019 field translocations.	Cliffs/BGPA	Information obtained on the germination, survival and growth of translocated *Tetratheca erubescens*.	Complete (2020 report)	
	October - November	Population demographic survey	BGPA	Obtain annual population demographic information for *Tetratheca erubescens*.	Complete (2020 report)	
2020	March - April	Detailed design of 2020 field translocations.	BGPA/Cliffs	Approved translocation proposal for 2018 translocations.	Complete	
	April	Annual reporting	Cliffs	Report to OEPA and DBCA on results and progress against plan.		
	April - May	Monitoring of 2019 field translocations.	Cliffs/BGPA	Information obtained on the germination, survival and growth of translocated *Tetratheca erubescens*.	Completed (2021 report)	
	May - June	Implementation of 2020 field translocations.	BGPA/Cliffs	Translocations established as per translocation design.	Completed (2021 report)	
	June 2020 – March 2021	Maintenance of 2020 field translocations.	Cliffs/BGPA	Information obtained on the germination, survival and growth of translocated *Tetratheca erubescens*.	Completed (2021 report)	
	October 2020 - February 2021	Root rhizosphere soil investigations and *Tetratheca erubescens* tissue analysis.	BGPA	Information on soil characteristics and *Tetratheca erubescens* nutrien acquisition strategies within translocation populations.	Completed (2020 report)	Section 3 pp. 58-62
	October – November	Monitoring of 2017, 2018, 2019 and 2020 field translocations.	Cliffs/BGPA	Information obtained on the germination, survival and growth of translocated *Tetratheca erubescens*.	Completed (2021 report)	



	October - November	Population demographic survey	BGPA	Obtain annual population demographic information for *Tetratheca erubescens*.	Complete (2021 report)	Section 2 pp. 28-34
2021	April	Annual reporting	Cliffs	Report to OEPA and DBCA on results and progress against plan.		
	April - May	Monitoring of 2020 field translocations.	Cliffs/BGPA	Information obtained on the germination, survival and growth of translocated *Tetratheca erubescens*.	Complete	
	July - August	Molecular analysis of soils within *Tetratheca erubescens* translocation sites.	BGPA	Soil biota characterisation within translocation populations and comparison with the population in natural sites.	Updated (2022 report)	P1 Section 3 pp. 27
	October - November	Monitoring of 2017, 2018, 2019 and 2020 field translocations.	Cliffs/BGPA	Information obtained on the germination, survival and growth of translocated *Tetratheca erubescens*.	Complete (2022 report)	P2 Section 2 pp. 36-64
	October - November	Population demographic survey	BGPA	Obtain annual population demographic information for *Tetratheca erubescens*.	Complete (2022 report)	P2 Section 2 pp. 48-52
	November - December	Complete population demographic modelling.	BGPA	Long-term population demographic model for *Tetratheca erubescens*.	Complete (2022 report)	P2 Section 2 pp. 55-61
2022	April	Prepare and submit a final report providing the results and outcomes of the Stage 1 *Tetratheca erubescens* Offsets Plan.	Cliffs/BGPA	Summary report capturing the results and outcomes of the five-year offsets plan.	Complete (2022 report)	Final Report 106 pgs.
	April - May	Undertake a review of the offsets plan in consultation with OEPA and DBCA and prepare and submit a revised offsets plan, if required.	Cliffs	Results of the five-year offsets plan considered in detail and decisions made around the needs and content of a revised offsets plan. A revised offsets plan developed and submitted to OEPA for approval, if required.		

APPENDIX 2

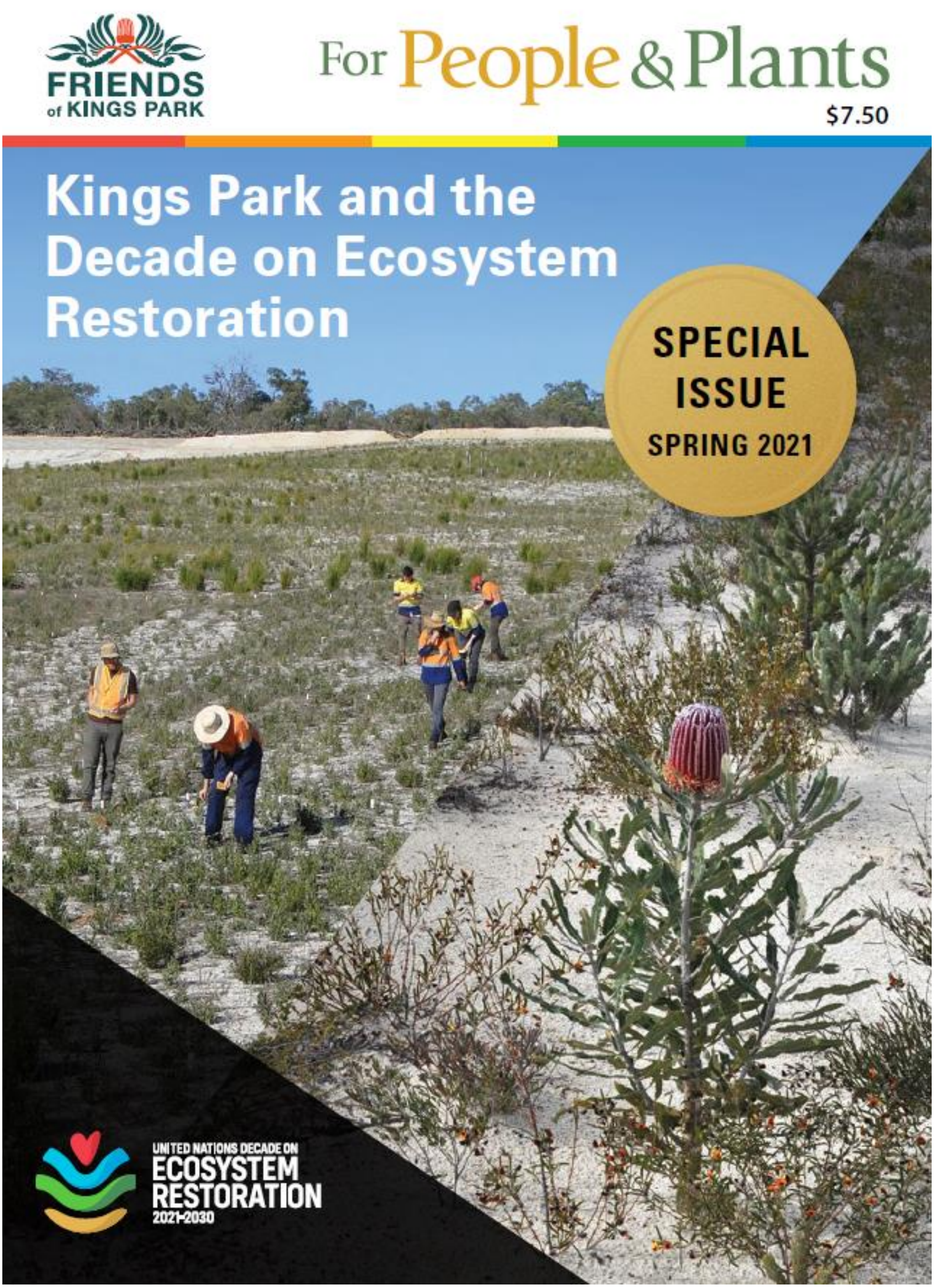
Publications, conferences, workshops, requested reports or project publicity associated with the research program.

Table S2.1. Date, type of activity and details of activity that relates to the publicity of the *Tetratheca* research program (2021-2022 period only).

Date	Activity	Details
April 2021	Conference: Australasian Seed Science	"Born to rock: seed traits of rare and common banded ironstone species from semi-arid ecosystems"
May 2021	Publicity: ABC Radio National	Interview on rare species in Western Australia and the research on *Tetratheca erubescens*
July 2021	Short report: for Goldfields Threatened Species Recovery Team	Update on the monitoring of *Tetratheca erubescens* and *Ricinocarpos brevis* translocations
Sept 2021	Publication: in 'For People and Plants' Special Issue - UN Decade on Ecosystem Restoration	"Life on the edge – translocation of rare cliff-dwellers" (pg. 32-33.)
Sept 2021	Presentation: for Botanic Gardens Spring Festival audience	Science seminar on *Tetratheca erubescens* research presented to the public as part of the Kings Park Festival.

Table S2.2. Summary of communication activities for the research program (2017-2022; see Annual Reports 1-4: Elliott *et al*. 2018-2021).

Activity	Number
Conference	2
Presentation	4
Publication	6
Publicity	2
Short report	8
Workshop	1



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For People & Plants

$7.50

Kings Park and the Decade on Ecosystem Restoration

SPECIAL ISSUE
SPRING 2021

UNITED NATIONS DECADE ON
ECOSYSTEM
RESTORATION
2021-2030

Life on the edge

Translocation of rare cliff-dwellers

Dr Wolfgang Lewandrowski Research Scientist
Dr Carole Elliott Research Scientist

Tetratheca erubescens in full bloom, wedged into a banded ironstone cliff, Koolyanobbing Range. Photo: Wolfgang Lewandrowski

Location, location, location! Stunning views over an ancient, eroded landscape!

Tetrathecas from the Goldfields of Western Australia are a unique group of plants living life on the top of craggy, ironstone ridges.

These small iron-loving shrubs are often locally abundant, but endemic to a single or few ridges. Several Tetratheca species are rare or endangered and require restoration post mining.

Translocation is critical to the restoration of Tetratheca establishing new populations into new sites away from continuing threats or degrading processes.

Threatened and rare plant species often have highly-specialised traits that enable them to live in extreme environments. However, these pose significant challenges to their successful restoration.

Building on 15 years of threatened species research on Banded Ironstone Formations (BIF) in Western Australia, we embarked on an integrated approach to inform the translocation of the rare *Tetratheca erubescens* J.P.Bull. This prior history into species distribution models, fine-scale habitat assessment, genetic diversity and structure, plant-water relations, and pollination of Tetrathecas were critical for planning translocations.

Tetratheca erubescens is a shrub with only 6,300 individuals covering 3.5 ha over a 1.6 km range exclusively on BIF on the Koolyanobbing Range. It grows out of rock cracks, fissures or holes on rocky outcrops or cliff faces. Recent mining removed around 900 plants, with a Ministerial Condition mandating to '*establish a new self-sustaining population … on a suitable landform that is suitable for the species*'. Four experimental translocations were established between 2017 and 2020.

Habitat modelling showed *T. erubescens* was able to grow best where it accessed water-catchments and climatic refuges within the rock. Banded ironstone stores moisture which plants can access in holes that are a part of a complex network of cracks and fissures. This knowledge was critical to finding locations for new plants and led to higher survival in translocation sites.

Assessment of genetic diversity and relationships between geographically separated *T. erubescens* populations were critical to inform seed collection zones and sampling of cuttings for propagation. Tube stock and seeds have been planted across five experimental translocation sites in pre-drilled rock holes for four years.

A range of horticultural treatments and field designs were trialled to determine optimal growth and performance. Seeds are generally considered the most cost-effective means to restore at scale, so understanding the conditions that trigger seed germination and seedling emergence is important.

Tetratheca erubescens seedling emerges from a drill hole. Seeds were treated to alleviate dormancy. Germination, emergence and survival were monitored for the lifetime of the seedlings. Photo: Wolfgang Lewandrowski

Ecophysiological assessments of *Tetratheca erubescens* using a gas exchange analyser to determine photosynthetic rates and fluorescence. Photo: Wolfgang Lewandrowski

Translocated *Tetratheca erubescens* cutting flowering and surviving in a drill-hole on banded ironstone formations. Photo: Wolfgang Lewandrowski

The translocation sites are being monitored regularly to follow plant survival, health and reproduction, compared to natural populations. This includes ecophysiological measurements that provide a useful tool to quantify stress-responses and key for understanding plant physiological function relative to their environment.

Our greatest challenge is understanding below ground processes: how plant roots grow and access water through cracks and crevices within the rock.

The success of the *T. erubescens* research project is attributed to inter-disciplinary collaborations and links between science and practice. More than 100 scientists, practitioners and volunteers have made contributions.

The project has also provided opportunity to train the next generation of restoration scientists and practitioners through engagement with universities, volunteer groups and the community. Our outreach includes volunteer opportunities, research projects and on-ground engagement with industry. We look forward to what the future of this project holds and continue to engage with passion and persistence to improve our ability to translocate rare species that inhabit these extreme environments. ■

4 experimental translocations installed
3057 tube stock planted
6000 seeds sown
100+ people involved

APPENDIX 3

Table S3.1: Understanding the context of translocation efforts on banded ironstone – A generalised snapshot that omits experimental complexity.

Species	Location	Habitat	Research program	Start year	Irrigated	No. seed	No. seedlings	No. survivors	No. greenstock	No. survivors	Main Reference
Tetratheca erubescens	Koolyanobbing	Natural (cliff)	Extensive (5 year)	2017	N	6000	151 (2.5%)	4 (2.6%)	3072	87 (2.8%)	1
Tetratheca paynterae subsp. *paynterae*	Windarling	Natural (cliff)	Limited (1 year)	2004	N	800	16 (2%)	8 (50%)	-	-	2
		Natural (cliff)	Limited (1 year)	2014	N	800	0	-	-	-	3
Ricinocarpos brevis	Windarling	Waste Rock	Extensive (5 year)	2014	Y	7000	250 (3.6%)	<200 (<75%)	380	185 (65%)	4
Darwinia masonii	Mount Gibson	Drill pad	Moderate (3-4 year)	2005	Y	-	-	-	209	168 (81%)	5
					N	-	-	-	20	0	
		Natural (suitable slope or ridge)	Extensive (5 year)	2009	N	-	-	-	195	74 (38%)	6 & 7
		Natural (unsuitable sandplain or clay plain)			N	-	-	-	390	45 (12%)	
Lepidosperma gibsonii	Mount Gibson	Natural (suitable slope or ridge)	Extensive (5 year)	2009	N	-	-	-	390	238 (61%)	6 & 7
		Natural (unsuitable sandplain or clay plain)			N	-	-	-	390	35 (9%)	
Acacia cerastes	Mount Gibson	Natural (suitable slope or ridge or sandplain)	Moderate (3-4 year)	2009	N	-	-	-	585	562 (96%)	7
		Natural (unsuitable clay plain)			N	-	-	-	195	27 (14%)	

DATA SOURCE: Australian Plant Translocation Database | Australian Network for Plant Conservation (anpc.asn.au) (Silcock *et al*. 2019).

Appendix 3: References

1. Elliott C, Lewandrowski W, Turner S, Krauss S, Merritt D, Miller B, Stevens J (2022) *Tetratheca erubescens* Translocation Final Research Report. Prepared by Kings Park Science, Biodiversity and Conservation Science in the Department of Biodiversity, Conservation and Attractions for Mineral Resources Ltd.
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3. Botanic Gardens and Parks Authority (2015) *Tetratheca paynterae* subsp. *paynterae* Research trial translocation – Final report. Prepared by Botanic Gardens and Parks Authority for Cliffs Asia Pacific Iron Ore Ltd.
4. Elliott, Turner and Miller (2018) Restoration Research Final Report (2013-2018): *Ricinocarpos brevis* (Euphorbiaceae). Prepared by Botanic Gardens and Parks Authority for Cliffs Asia Pacific Iron Ore Ltd.
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6. Miller and Barrett (2010) *Darwinia masonii* and *Lepidosperma gibsonii* conservation and restoration research. Prepared by Botanic Gardens and Parks Authority for Mount Gibson Mining Ltd and Extension Hill Pty Ltd.
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