Department of **Biodiversity, Conservation and Attractions**

GOVERNMENT OF WESTERN AUSTRALIA

Biodiversity and Conservation Science

BOTANIC GARDENS & PARKS AUTHORITY

KINGS PARK SCIENCE

ABN 30 706 225 320

Tetratheca erubescens Translocation Annual Research Report 4

for Mineral Resources Limited

March 2020 to March 2021

Dr Carole Elliott, Research Scientist
E: Carole.Elliott@dbca.wa.gov.au

Dr Wolfgang Lewandrowski, Research Scientist
E: Wolfgang.Lewandrowski@dbca.wa.gov.au

Dr Shane Turner, Senior Research Scientist
E: Shane.Turner@dbca.wa.gov.au

Dr Siegy Krauss, Senior Research Scientist
E: Siegy.Krauss@dbca.wa.gov.au

Dr David Merritt, Senior Research Scientist
E: David.Merritt@dbca.wa.gov.au

Dr Ben Miller, Senior Research Scientist
E: Ben.Miller@dbca.wa.gov.au

Dr Jason Stevens, Senior Research Scientist
E: Jason.Stevens@dbca.wa.gov.au
Tel: 9480 3614

KINGS PARK SCIENCE
1 Kattidj Close, Kings Park
Western Australia 6005
www.bgpa.wa.gov.au

EMAIL: scienceadmin@dbca.wa.gov.au
Telephone: +61 8 9480 3614
Facsimile: +61 8 9322 3641

TABLE OF CONTENTS

Please cite this document under the following:

Elliott C, Lewandrowski W, Turner S, Krauss S, Merritt D, Miller B, Stevens J (2021) *Tetratheca erubescens* Translocation Annual Research Report 4. Prepared by Kings Park Science, Biodiversity and Conservation Science in the Department of Biodiversity, Conservation and Attractions for Mineral Resources Ltd.

EXECUTIVE SUMMARY

Kings Park Science's "*Tetratheca erubescens* Translocation Research Program" commenced in June 2017 and has been active for <4 years. The broad research objectives of the 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) to assess functional attributes within restored / translocated populations to determine their long-term sustainability compared with natural populations.

This report highlights new research findings (March 2020 – March 2021) and summarises ongoing research since project commencement (June 2017). The following has been achieved:

- Program 1: Seed Biology
    - Completed priming approaches to enhance germination speed under controlled conditions.
    - Completed investigation into seed enhancement design (using priming and pelleting technology).
- Program 2: Translocation and monitoring
    - Conducted ongoing monitoring events of seedling emergence and greenstock survival in five translocation sites that were established in 2017 and replanted in 2018 and 2019.
    - Established five translocation trial sites in 2020 that involved *in situ* placement of 900 seed and 720 nursery propagated greenstock.
    - Monitored natural demographic sites four times to assess plant growth, health, ecophysiology and fecundity of adults, juveniles and seedlings.
- Program 3: Plant function, habitat and substrate interactions
    - Continued ecophysiological measurements of plant function in natural populations (four sites) with a focus on *Tetratheca erubescens* and co-occurring BIF species.
    - Compared plant function in natural and translocation sites with a focus on slope aspect of populations.
    - Continued storage of soils for molecular analysis to understand niche level microbial processes underpinning ecosystem function.
    - Continued soil temperature and moisture data collection in three natural and two translocation sites to monitor environmental conditions.

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 individuals 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 a number of specific challenges for restoration and translocation of populations. Effective, sustainable translocation of plant individuals 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 aims to:

- Develop methods to support the translocation and restoration 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 suitable 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 outlines the progress and outcomes of the scientific approach from March 2020-March 2021 that aims to:

- Develop practical, effective and sustainable restoration of *Tetratheca erubescens.* This will be achieved through understanding and optimising their establishment ecology and environmental requirements.
- Determine how these can be effectively utilised or recreated in restored systems. Thus ensuring 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 is 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 is 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 involve:

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 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 to support practical, effective and sustainable restoration of *Tetratheca erubescens* through investigation in three key disciplines: seed biology and enhancement (Program 1); translocation and demographic studies (Program 2) and plant-substrate interactions (Program 3).

RESEARCH RESULTS

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:

- Optimal temperature for germination was between 15 - 20°C (even after breaking dormancy through warm stratification).
- Alternating temperatures (e.g. 20/10°C, or 25/15°C) did not support germination.
- There is no difference between alternating light/dark and constant dark conditions for seed germination.
- Details of research are in Annual Research Reports 1-3 respectively (Elliott *et al.* 2018; Elliott *et al.* 2019; Elliott *et al.* 2020).

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

Research outcomes:

- Seeds require at least 14 days of optimal water availability (0 to -0.25 MPa) for germination.
- 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.
- 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).

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

Research outcomes:

- Highest germination was achieved by stratifying seeds for 6 weeks.
- Application of a germination stimulant (KAR_1) further increased germination responses by 10-15%.
- Application of stratification treatment to water stress experiments demonstrate an increased capacity for seeds to germinate at lower water availabilities.
- Details of research are in Annual Research Reports 1 and 2 respectively (Elliott *et al.* 2018; Elliott *et al.* 2019).

1.1.4 Define the role of germination stimulants in promoting germination.

Research outcomes:

- The smoke related germination stimulant karrikinolide (KAR_1) significantly improves germination at temperatures between 10-20°C in fresh seeds and during dormancy loss following stratification, after-ripening and wet/dry cycling.
- KAR_1 increases the capacity for seeds to germinate into water stress (Section 1.1.2).
- Details of research are in Annual Research Reports 1 and 2 respectively (Elliott *et al.* 2018; Elliott *et al.* 2019).

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.

Current research outcomes:

- Hydro- and osmo-priming seeds for 8 days increases germination speed compared to fresh seeds; fastest germination was recorded after 16.6 and 17.3 day respectively.
- Osmopriming after 8 days demonstrated highest germination of up to 80%.
- Despite increasing germination speed, there was no advantage for optimally hydro- and osmo-primed seeds under water stress conditions, when compared to stratified seeds.

Priming involves hydrating seeds sufficiently to advance the metabolism involved in germination without the seed germinating, followed by drying of the seed (Hardegree and Emmerich 1992; Bewley *et al.* 2013). Primed seeds generally demonstrate a more synchronous and faster germination than non-primed seeds. To determine the effects of priming, 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. Hydropriming involves hydrating seeds in water for different periods of time, while osmopriming, involves hydrating seeds in solutions containing different water potentials to regulate maximum water uptake. A water potential of -1.0 MPa was selected as the water potential threshold in the osmotic solution (as determined from experiments conducted in Section 1.1.2; Elliott *et al.* 2020), and prepared by dissolving polyethylene-glycol (PEG-8000, Sigma-Aldrich Pty. Ltd., Sydney, NSW, Australia) in water following the methodologies outlined in Michel (1983). For both methodologies, a priming time experiment was conducted that tested different hydration time durations. The hydration period for both methodologies was 0 (control), 4, 8 and 12 days. We did not test for longer periods, as we have previously observed germination in dormancy alleviated seeds after 14 days (see Section 1.3.3; Elliott *et al.* 2020) and thus risk germinating seeds during the priming process.

The seed treatments tested were untreated seeds that were freshly collected (Control – fresh), dormancy alleviated seeds that were stratified (Control - stratified), and dormancy alleviated seeds (through stratification) that were either hydroprimed, or osmoprimed at the

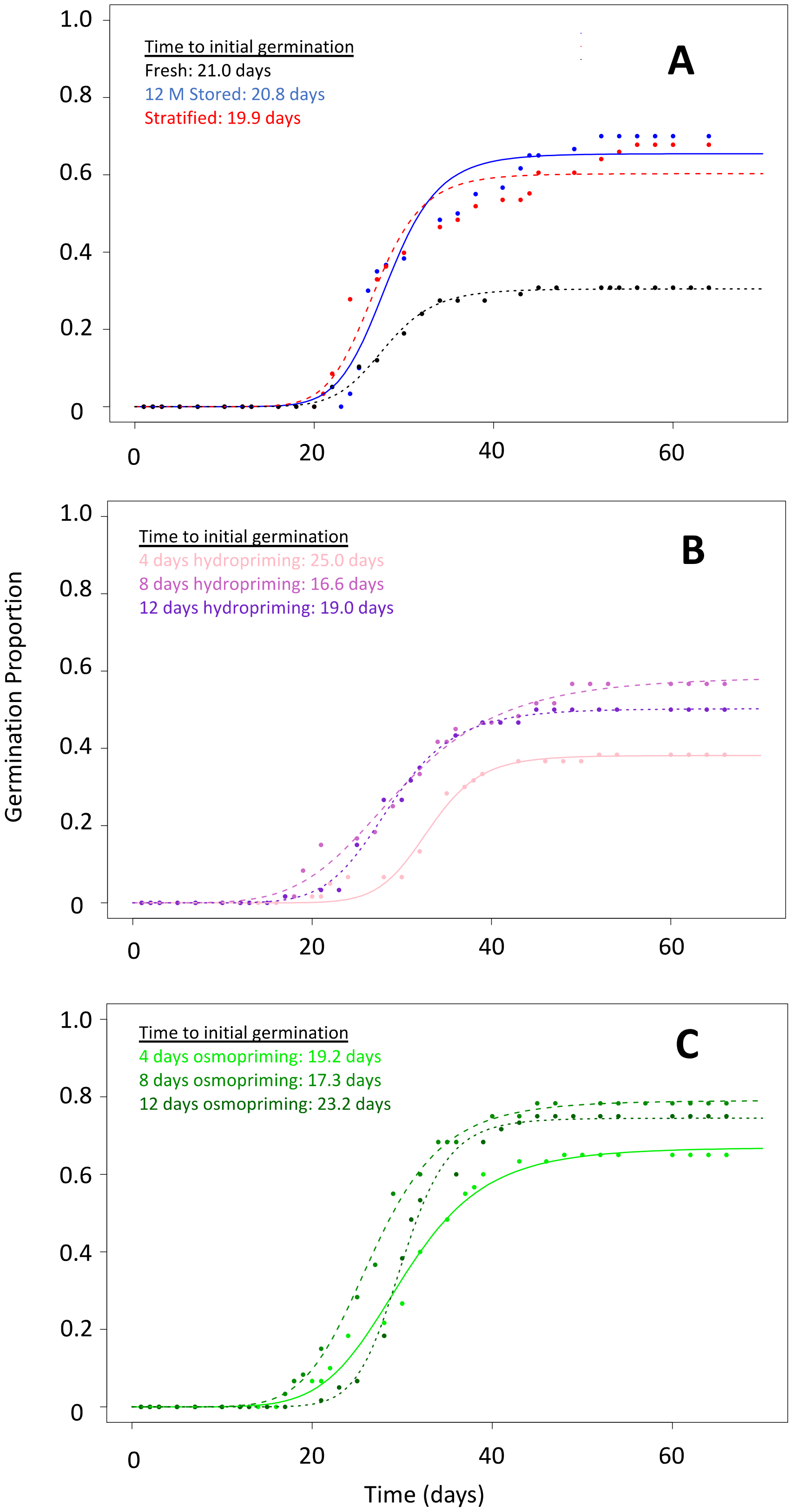


Figure 1.2.1a. Proportion of seeds to germinate over 65 days for A) Control: fresh, 12 months stored or stratified seeds; B) hydroprimed seeds; and C) osmoprimed seeds. The two priming treatments occurred over a period of 4, 8 and 12 days.

different hydration times. We also included a comparison against seeds that were stored for 12 months at 15% relative humidity and at constant 15°C (Control – 12M stored).

Initial germination was recorded after 21 days from fresh seeds with maximum germination proportions that never exceeded 30% (Figure 1.2.1a). Seeds that were stratified or stored for 12 M, germinated to higher proportions (60-65%, respectively), and marginally quicker for initial germination responses (Figure 1.2.1a.a). Hydro- and osmo-primed seeds demonstrated an increase in germination speed as the hydration period increased from 4-8 days, while treatment of seeds for 12 days generally slowed germination (Figure 1.2.1b.c). Fastest germination was recorded after 16.6 and 17.3 days, in hydro- and osmo-primed seeds that were hydrated for 8 days, respectively (Figure 1.2.1b.c). Despite increasing germination speed after 8 days of treatment, the hydroprimed seeds only germinated to 60%, while the osmoprimed seeds germinated to 80%.

Hydro- and osmopriming treatments after 8 days represented the optimal priming treatments and their germination responses were compared on a water stress gradient against the control treatments (e.g. Control: fresh, 12M stored and stratified seeds). A water stress gradient was created by using thermally corrected polyethylene-glycol solutions (PEG-8000, Sigma-Aldrich Pty. Ltd., Sydney, NSW, Australia) following the methodologies outlined in Michel (1983). The seed treatments were incubated in the different osmotic solutions for 60 days. Along a water stress gradient 0 MPa represents freely available water and is usually associated with optimal and non-limiting moisture conditions, while at -1.5 MPa seeds are exposed to conditions representing water limited conditions and the permanent plant wilting point (Bewley *et al*. 2013; Bradford 2002).

Germination decreased under higher water availabilities for both hydro- and osmo-priming treatments (Figure 1.2.1b; Table 1.2.1a). The sensitivity thresholds limiting germination were consistently higher for both priming treatments, when compared to the fresh, 12M stored, and stratified seeds. This response could be explained by priming inducing conditional dormancy, whereby seeds would have a high response under narrow optimal conditions, however, decrease rapidly into limiting environmental conditions (Baskin and Baskin, 2014). Interestingly, despite germinating at lower proportions, the dormant fresh seeds were demonstrating wider germination thresholds into water stress than either of the priming treatments. The wide germination thresholds for the 12M stored and stratified seeds were likely as result of the seeds being in a lower dormancy condition because of the prolonged storage period for the 12M stored seeds, or the 6-week stratification treatment. For all treatments, cut-testing seeds and tetrazolium staining indicated that seeds were still viable at the completion of the water stress-experiment.

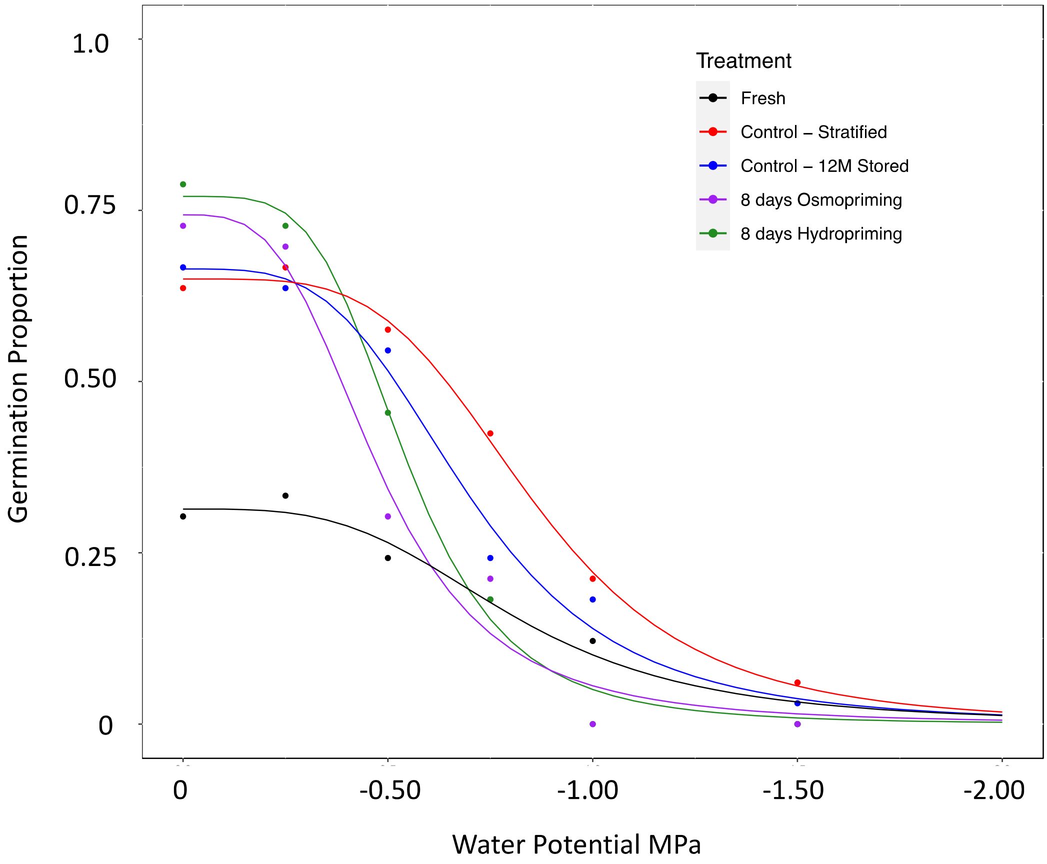


Figure 1.2.1b. Germination responses to water stress after 60 days from fresh, stratified, 12 M stored, osmoprimed and hydroprimed. The seeds were incubated on a water potential gradient mimicking freely available water (e.g. 0 MPa) and high-water stress (e.g. -1.5 MPa).

Table 1.2.1a. Water stress thresholds limiting germination at the median response, and at the 90th percentile response. The median represents the thresholds reducing germination by 50%, while at the 90th percentile, germination responses are close to zero, representing the tail-end of the response curve.

Treatment	Ψb50 [MPa]	Ψb90 [MPa]
Control - Fresh	-0.69 ± 0.04	-1.26 ± 0.12
Control -12 M stored	-0.85 ± 0.04	-1.44 ± 0.16
Control - Stratified	-0.81 ± 0.12	-1.51 ± .0.33
Osmopriming for 8 days	-0.47 ± 0.03	-0.91 ± 0.08
Hydropriming for 8 days	-0.54 ± 0.03	-0.90 ± 0.07

Applications:

- While priming increases maximum germination and speed responses, there is no apparent benefit under water stress conditions that is a limiting factor under field conditions.
- As the current tested priming-methodology is likely to induce conditional dormancy (e.g. high germination under narrow optimal conditions) for *T. erubescens* seeds, we do not recommend this treatment for field sowing or translocation trials.

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

Research outcomes:

- The pelleting techniques were not suitable for *T. erubescens*, due to loss of seed during the process of producing small pellets and the impracticality of finding suitably sized cracks to accommodate a larger pellet.
- Details of research are in Annual Research Reports 2 and 3 respectively (Elliott *et al.* 2019; Elliott *et al.* 2020).
- 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*.

As pelleting was previously reported to impede seedling emergence, we tested a 'slurry' soil matrix, which also had the capacity to deliver the beneficial microbes, nutrient and growth factors (e.g. increased water holding capacity) to promote *in situ* germination, emergence and seedling establishment. We constructed a brick, gravel and topsoil substrate as our artificial propagation structure under controlled conditions (cool room at 20°C with daily watering of 6ml) and used *T. erubescens*, *Acacia hilliana* and *Androcalva perlaria* seed to test a 'slurry' soil matrix (Figure 1.2.2a). Emergence of two species (*T. erubescens*, *A. hilliana*) occurred in both the control (topsoil only) and enhanced (topsoil, water holding crystals) soil matrices. Emergence started 25 days (*A. hilliana*) post-sowing and the final emergence was observed at 80 days (*T. erubescens*; experiment terminated at 22 weeks). Emergence was 3.3-5%, for *T. erubescens* and *A. hilliana* respectively, and was inconclusive regarding the enhanced soil matrix. All seedlings survived the construct and 'slurry' environment until termination. Seedlings were harvested at the end of the experiment and rooting structure was found to reach the base of the artificial construct under both soil matrices.

The 'slurry' settled into a hard setting substrate for both the control and enhanced soil matrices, potentially impacting on emergence. This has been observed in similar studies and alterations to the mix to include a sand or loam substrate into the recipe improved overall emergence (Stock et. al. 2020). Altering the soil matrix to include a sand or loam would be the next stage of testing the 'slurry' efficacy. During the course of the experiment, we observed a salt build up on the surface of the bricks that may have affected the later part of the experiment. The artificial construct was suitable to test the effect of a 'slurry' matrix, but the materials used need to be examined for secondary compounds like salt exudate.

The feasibility of applying a 'slurry' soil matrix using an artificial propagation structure was positive, however, the slow and low levels of emergence of *T. erubescens* and *A. hilliana* do not support the use of this approach in the field. The emergence from the 'slurry' soil matrix indicates this approach does not improve responses beyond that observed in *in situ* translocation direct sowing (0.2-3.5% under ambient conditions; Elliott *et al.* 2019 and Elliott *et al.* 2020) or under *ex situ* germination conditions (50-80% under optimal laboratory conditions; see Section 1.2.1) and is not a recommended approach at this stage.

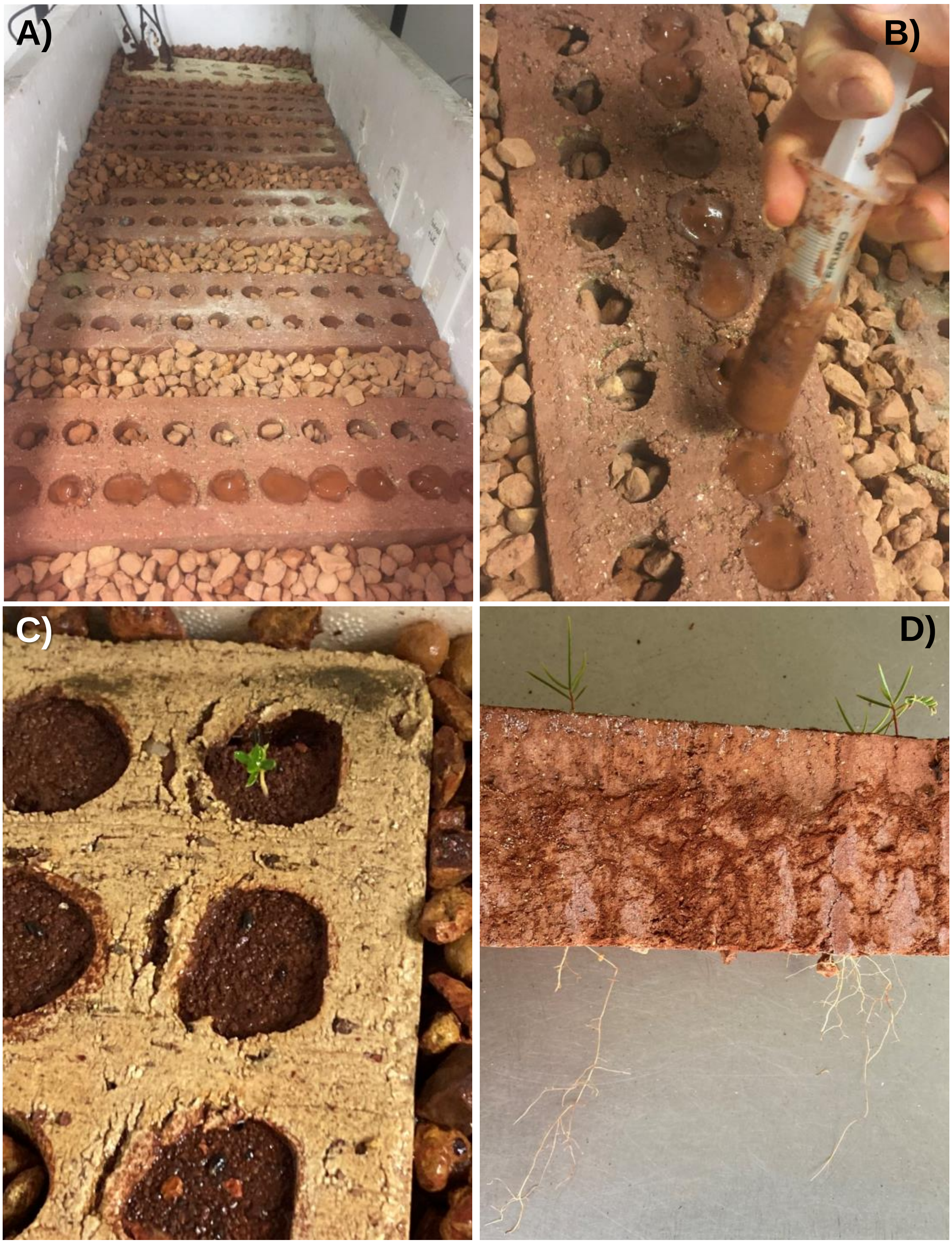


Figure 1.2.2a. 'Slurry' soil matrix trial: A) artificial propagation structure used to test treatments; B) application of 'slurry' soil matrix into structure; C) *T. erubescens* seedling that emerged in 'slurry'; and D) size of *A. hilliana* seedlings at harvest. Images A. Ritchie.

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.

Current research outcomes:

- Seedling emergence was observed in August 2020 (total of 14 seedlings).
- This emergence was lesser than August 2018 (29 seedlings) or 2019 (72 seedlings); possibly due to the average June and July rainfall in 2020.
- There was no seedling survival after the summer period (2020/2021); possibly due to average, but late, climate conditions in spring and summer.
- Only the oldest two seedlings, recruited in 2018 and 2019, were still alive after the 2020/2021 summer.
- No collection of fresh seed occurred due to low levels of flowering and fruiting.

Translocations – 2017-2020

The habitat characteristics and results from the 2017-2020 translocation sites are summarised in Table 2.1.1a. 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 2.1.1a).

Details of these locations and the overall numbers of seed and greenstock trialled at each location for the 2020 translocation is summarised in Table 2.1.1b. Further details of the treatment design for the direct seeding are in Table 2.1.1g and for the greenstock planting are in Table 2.1.1h. Details of the 2017-2019 translocation trials are in Annual Research Reports 1-3 respectively (Elliott *et al.* 2018; Elliott *et al.* 2019; Elliott *et al.* 2020).

Table 2.1.1a. Summary of specific 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 2.1.1b. Summary of the number of seed sown and greenstock planted for each translocation site (2020).

Site	Latitude	Longitude	Seed sown	Greenstock
T6	-30.87245	119.60269	300	160
T18	-30.88656	119.61919	300	290
T21	-30.87394	119.60513	-	90
T23	-30.87150	119.60637	-	50
T24	-30.87417	119.61111	300	130
Total			900	720

Monitoring schedule for 2017-2020 translocations were as follows:

Table 2.1.1c. Summary of installation and monitoring periods for each translocation site (Translocation 2017). Translocation site T19 only had the first monitoring assessment and none afterwards (see above).

		Monitoring											
Site	**Installed**	**1st**	**2nd**	**3rd**	**4th**	**5th**	**6th**	**7th**	**8th**	**9th**	**10th**	**11th**	**12th**
T6	9-16th Aug 2017 Late Winter	18-25th October 2017 9 wks	8-15th February 2018 25 wks	16-23rd August 2018 51 wks	21-28th October 2018 61 wks	12-20th February 2019 77 wks	22-30th August 2019 97 wks	30-7th November 2019 107 wks	14-26th February 2020 121 wks	11-20th May 2020 133 wks	14-21st August 2020 149 wks	23-28th October 2020 158 wks	22-2 February 2021 175 wks
T18													
T19													
T21													
T23													

Table 2.1.1d. Summary of installation and monitoring periods for each translocation site (Translocation 2018).

		Monitoring									
Site	**Installed**	**1st**	**2nd**	**3rd**	**4th**	**5th**	**6th**	**7th**	**8th**	**9th**	**10th**
T6	14-21st June 2018 Early Winter	16-23rd August 2018 8 weeks	21-28th October 2018 18 weeks	12-20th February 2019 34 weeks	22-30th August 2019 54 weeks	30-7th November 2019 64 weeks	14-26th February 2020 78 weeks	11-20th May 2020 90 weeks	14-21st August 2020 106 wks	23-28th October 2020 115 wks	22-2 February 2021 132 wks
T18											
T21											
T23											
T24											

Table 2.1.1e. Summary of installation and monitoring periods for each translocation site (Translocation 2019).

		Monitoring						
Site	**Installed**	**1st**	**2nd**	**3rd**	**4th**	**5th**	**6th**	**7th**
T6	13-23rd Jun 2019 Early Winter	22-30th Aug 2019 8 weeks	30-7th Nov 2019 18 weeks	14-26th Feb 2020 32 weeks	11-20th May 2020 44 weeks	14-21st Aug 2020 60 weeks	23-28th Oct 2020 69 weeks	22-2 Feb 2021 86 weeks
T18								
T21								
T23								
T24								

Table 2.1.1f. Summary of installation and monitoring periods for each translocation site (Translocation 2020).

				Monitoring		
Site	**Latitude**	**Longitude**	**Installed**	**1st**	**2nd**	**3rd**
T6	-30.87245	119.60269	16-24th June 2020 Early Winter	14-21st August 2020 7 weeks	23-28th October 2020 18 weeks	22-2 February 2021 35 weeks
T18	-30.88656	119.61919				
T21	-30.87394	119.60513				
T23	-30.87150	119.60637				
T24	-30.87417	119.61111				

Table 2.1.1g. Summary of the 2020 translocation site, source of seed, treatment tested, location of seed line, number of seeds sown and the total number of seedlings emerged over the monitoring period (at 18 weeks; see Table 2.1.1f).

Site	Latitude	Longitude	Source of seed	Treatment tested	Seeding location	Number of seeds sown	Number of emergents
T6	-30.87245	119.60269	2017/2018	Stratified	Drill hole	160	0
					Fissure/crack	140	0
T18	-30.88656	119.61919	2017/2018	Stratified	Drill hole	130	0
					Fissure/crack	170	0
T24	-30.87150	119.60637	2017/2018	Stratified	Drill hole	160	0
					Fissure/crack	140	2
Total						**900**	**2**

Table 2.1.1h. Summary of the 2020 translocation site, source of greenstock and the total number of planted at each site.

Site	Latitude	Longitude	Greenstock source	Number planted June 2020
T6	-30.87245	119.60269	Cutting	80
			Seedling	80
T18	-30.88656	119.61919	Cutting	145
			Seedling	145
T21	-30.87394	119.60513	Cutting	45
			Seedling	45
T23	-30.87150	119.60637	Cutting	25
			Seedling	25
T24	-30.87417	119.61111	Cutting	65
			Seedling	65
Total				**720**

Direct seed sowing in 2017-2020 translocations

Seeds collected from natural plants in 2016/2017 (Elliott *et al.* 2017) were used in the direct seeding experiments in the 2017 and 2018 translocation trials. Due to limited seed availability from this initial collection, seeds for the 2019 and 2020 translocation trials were sourced from the 2017/2018 collection. The development and implementation of the translocation design for the *in situ* sown seeds, including the seed treatments, the number of replicates implemented, and the number of emergents counted within each translocation site, are summarised in Table 2.1.1g for the 2020 translocation.

Seeds that were sown in the 2020 translocation were placed within available natural cracks within the site and covered with topsoil (0.5-1cm) or drill holes, by placing topsoil halfway up the drill hole, sowing seeds and covering seeds with a layer of topsoil (~1cm). Each sowing location was visually inspected, and photos were taken when the locations were considered to have significantly changed due to disturbance (e.g. wash-out, run-off, or burial by vegetation) or when seedling status had changed. The seeding lines showed no evidence of washout 18 weeks after sowing. This indicated that natural rainfall during this period was not flushing soil from the 2020 seeding line locations.

Seedling emergence from our direct sowing lines was detected in August and October 2020, for all four years of direct seeding (2017: 1 seedling; 2018: 3 seedlings; 2019: 8 seedlings; 2020: 2 seedlings; for a total of 14 seedlings). This was a poorer emergence response than 2018 (29 seedlings in total; Elliott *et al.* 2019) or 2019 (72 seedlings in total; Elliott *et al.* 2020). Winter 2019 received 88.5mm of rainfall (1 June to 10 August), whereas the 2020 winter only received 68.8mm of rainfall over the same period, suggesting that the higher rainfall during the 2019 winter stimulated a greater germination response in all translocation trials. The continued emergence from seed that had been sown in previous years, despite a low recruitment pulse, demonstrates the persistence of seed in the soil seedbank after three years.

However, seedling survival after the summer period (2020/2021) for the 2020 recruitment was zero and there were further declines in survival of the previous year's recruitment events (recruitment in 2018: 1 seedling surviving three summers and 2019: 1 seedling surviving two summers; both at T21). Rainfall over summer 2020/2021 was 14% above average (1 Dec 2018 - 28 Feb 2021) however, the late summer rain on 27 Feb may have been too late for most seedlings (excluding this equates to 58% below average rain for 1 Dec – 26 Feb 2021; BOM, 2021). In addition to understanding that summer rains may be an important part of sustaining seedling survival from the 2018 emergence data, it is now apparent that the timing of these rainfall events is also critical to seedling survival in their first year, as the oldest seedlings (from 2018 and 2019) are still alive.

Future research:
Given the low survival from emergence lines, future research will aim to understand if the low survival is a result of the growth environment, or possibly a natural occurrence in the system. In order to address this issue, habitat characteristics will be measured on seedlings recruiting and surviving in natural sites, and compared with seedlings recruiting and surviving in translocation sites. A broad species comparison was undertaken during the 2019 translocation trial at T18, whereby a range of BIF species were sown into rock and ground strata. Data are currently being analysed and responses will be compared to *T. erubescens* germination and emergence results. Findings will be reported in the next annual report.

Fresh seed collections (2020/2021)

Tetratheca erubescens plants had a low level of fecundity during the 2020 flowering season and therefore, a seed collection event was not carried out for this season.

Future research:
Further investigation will be conducted on these seed collections to determine if there are additional environmental variables, such as genetic groups (as determined in Krauss and Anthony 2019), NE- and SW-facing aspects, or on a plot level to understand the variation in seed quality among locations on the ridge, in different years.

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

Research outcomes:

- It is feasible to derive greenstock from cuttings or seeds to establish plants in the field, based on the 2019 and 2020 translocations.
- Survival was similar between the two types of greenstock (2020 translocation).

Greenstock for the 2020 translocation trial that were derived from cutting material were sourced from stock plants (propagated in 2017) and maintained under controlled glasshouse conditions. Greenstock derived from seedlings were sourced from seedlings generated from laboratory trials or the 2018 seed collection. Cuttings and seedlings grown in biodegradable pots that were 8 months old at planting, both had well-developed stem biomass and visible root growth at the time of planting (Figure 2.1.2a). We planted 360 greenstock of each source type (seed or cutting derived) in the 2020 translocation across the five sites (Table 2.1.1h). Seedlings were able to be planted in the field and overall, we had similar survival of seedling derived greenstock as cutting derived greenstock in the early stages of the translocation, as rainfall was average (2% above) for the calendar year (see Section 2.2).

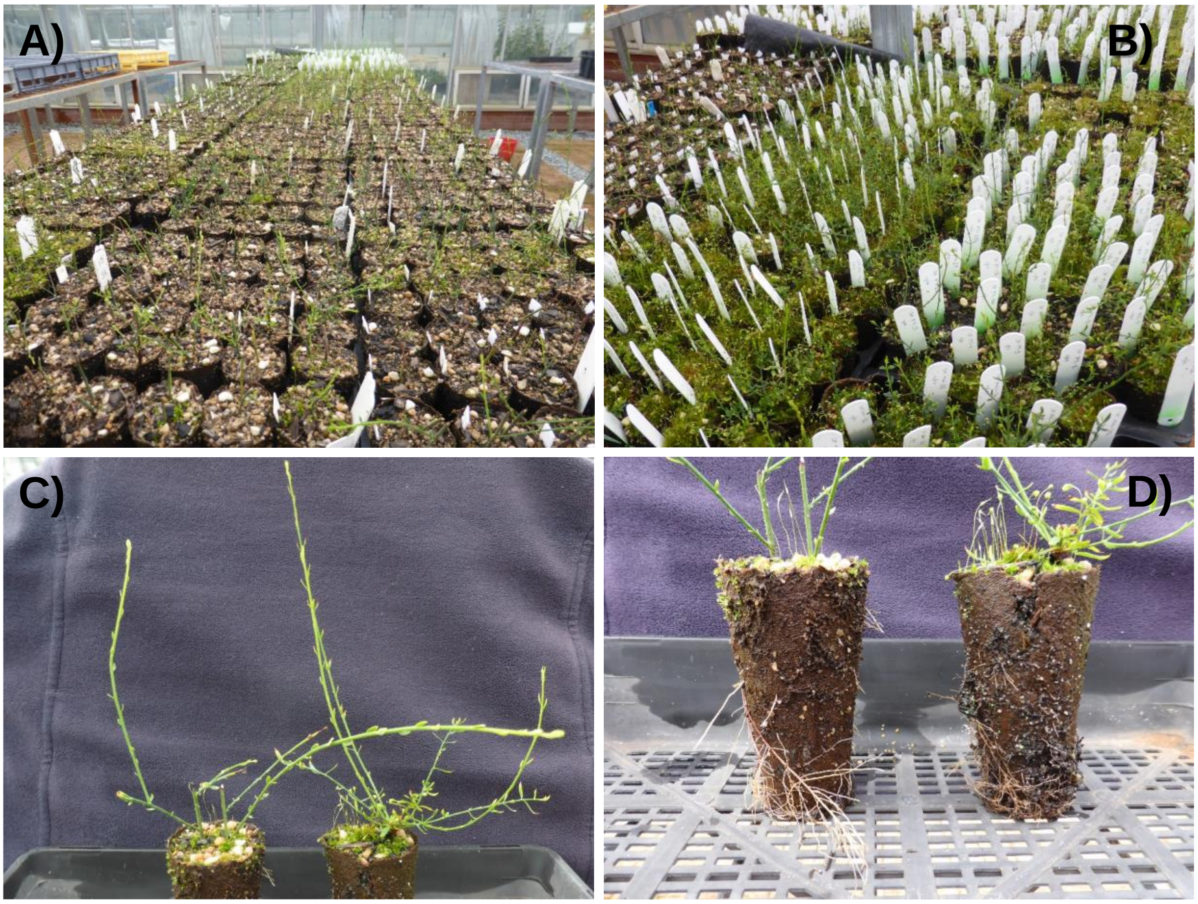


Figure 2.1.2a. Greenstock used in the 2020 translocation. A) seedling and cutting derived greenstock in biodegradable pots; B) seedling collection in glasshouse used in translocation; C) size of seedling (left) and cutting (right) greenstock in June 2020; and D) rooting status of seedling (left) and cutting (right) greenstock in June 2020. Images C. Elliott.

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

Current research outcomes:

- Greenstock locations were considered water capturing slope types and their survival was less variable in these water capturing locations.
- Preliminary review suggests that greenstock planting locations reflect the locations of natural plants (on local geomorphologic features and local slope type only).
- Natural recruitment and survival of seedlings was greatest in water capturing locations.
- Details of research are in Annual Research Reports 2 and 3 respectively (Elliott *et al.* 2019; Elliott *et al.* 2020).
- Peak soil temperatures were >60°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.
- Rainfall was approximately average in 2020 (2% above average).

Habitat characteristics

Review of planting locations

The choice of greenstock locations in the translocation sites reflected the breath of habitat characteristics where adult plants of *T. erubescens* occur (Miller 2015). The majority of greenstock were planted in water capturing locations (see Elliott *et al.* 2020), where naturally occurring plants were 2.4 times more likely to occur (Miller 2015).

Future research:

Kings Park Science will measure these habitat characteristics on individual seeding line locations within translocation sites in 2021 and any additional seedlings that emerge from recruitment events in the natural population, to determine if patterns of survival and growth can be better predicted by fine-scale assessment of individual sowing locations across multiple years.

Soil characteristics

Soil moisture and temperature loggers were installed in five sites (two translocation sites: T6 and T18; three natural sites: P5, P7 and P25) across northeast (NE) and southwest (SW) slope aspects (see Section 2.1.3 in Elliott *et al.* 2020 for installation details). Composite soil samples were collected from 0-5 cm depth at each site and used to determine soil water retention curves. The retention curves will help describe seed and plant available water calculations and site environmental effects on plant establishment and function in Programs 1, 2 and 3. Additionally, the retention curves help to described soil moisture availability ranging between field capacity (e.g. 100% = -0.01 MPa) and dry soil (e.g. 0 % < -10 MPa).

Across all sites, natural rainfall events of 5 mm were correlated with raising the soil water content to ~50% field capacity (Figure 2.1.3a), while rainfall events of 10 mm elevates the soil moisture content to >75% field capacity, though soil type, particle size and underlying substrate will influence moisture retention following rainfall. Higher soil moisture content was generally related to cooler periods (August- September), with higher soil temperature related with quicker soil drying events (e.g. November - January). During the warmer months, soil water content depleted to drier ranges 0-7% field-capacity (Figure 2.1.3a). The rainfall events that occurred in March 2021 (24 mm and 46mm on the 3rd and 4th respectively) raised the soil moisture to 68% field capacity and the moisture was retained in the soil profiles for up to 5 days.

A moisture window was consistently evident during autumn, winter and spring months (May - November) – where soil moisture levels were elevated (5-80%) and never completely dried (e.g. 0%). The increased moisture availability in winter was a likely result of lower evaporation rates evident from the soil profile due to cooler soil temperatures. Due to the lower total rainfall in 2019 and 2020 (212-271 mm, compared to >300 mm in 2017 and 2018), soil moisture availability was consistently lower across all sites during this period (Figure 2.1.3a and Figure 2.1.3b). For both translocation trials, planting and sowing seeds during this period coincided with the period of highest moisture availability. The spring period in 2019 and 2020 were both considered dry (see rainfall Figure 2.1.3b), with recently planted seeds, seedlings and cuttings exposed to prolonged periods without moisture recharge from rainfall. Interestingly, soil moisture peaks were higher in the translocation sites when compared to natural sites, however the moisture windows (the time moisture is available) were similar between the sites. A possible driver for the variation in the peaks could be the particle size composition of the substrate, surrounding vegetation shading the site and availability of hydrological zones in the BIF that may be retaining greater moisture than zones that are exposed.

There was a general cooling trend observed in some sites during summer (e.g. SW-aspect: Plot 5; NE-aspect: Plot 7 and 25; Translocation site: T18), with the soil temperatures quantified in 2017 (max temperature 56-67°C) consistently hotter compared to 2018, 2019 and 2020 (max temperatures 43-63°C). Despite the slight cooling trend, the sites were overall drier due to the decreasing rainfall trend. Natural populations with NE-facing aspects (P7 and P25) were consistently hotter (max temperatures: 55-65°C, Figure 2.1.3b) than SW-facing aspects (e.g. P5, max temperatures: 48-56°C; Figure 2.1.3b) between 2017 and 2021. In the translocation sites, T6 was consistently wetter than T18 (see Figure 2.1.3a), which is due to the majority of the site being shaded during the morning and having a SW-facing aspect. Despite having higher wetting profiles, T6 measured the highest soil temperatures during summer (65°C).

Future research:
Soil moisture and temperature logging will continue throughout the project to determine environmental requirements for natural and translocated *T. erubescens* populations. The data will also be correlated against ecophysiological measures. A full breakdown of moisture and temperature summary statistics will be available in the final report.

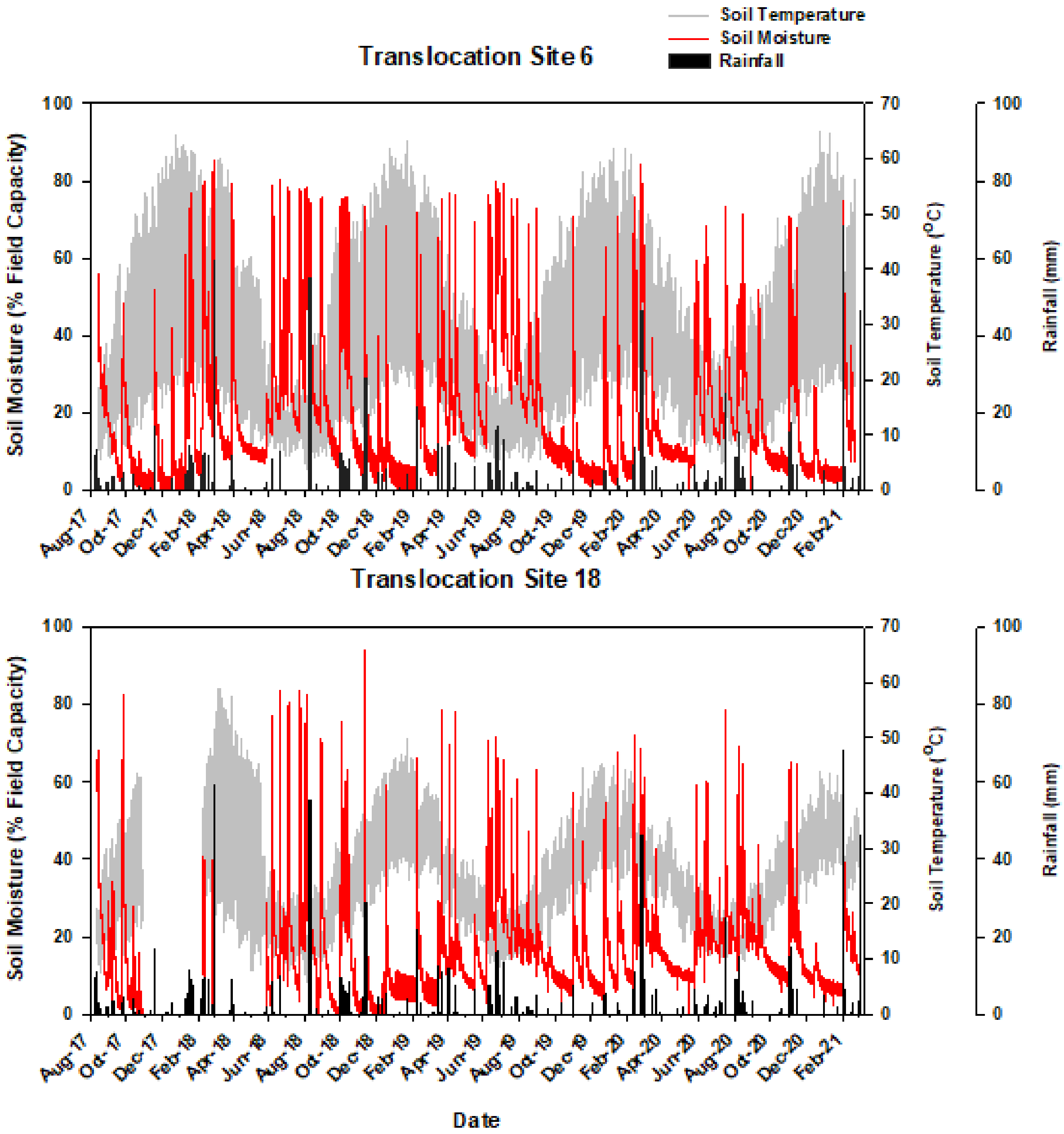


Figure 2.1.3a. Soil moisture, soil temperature and total daily rainfall for translocation sites T6 and T18 from August 2017 to February 2021. Soil moisture is shown as % Field capacity, with 100% indicating field capacity = -0.01 MPa, and dry 0% < -10 MPa. Rainfall data available from BOM, 2021 - Koolyanobbing, Site 12227.

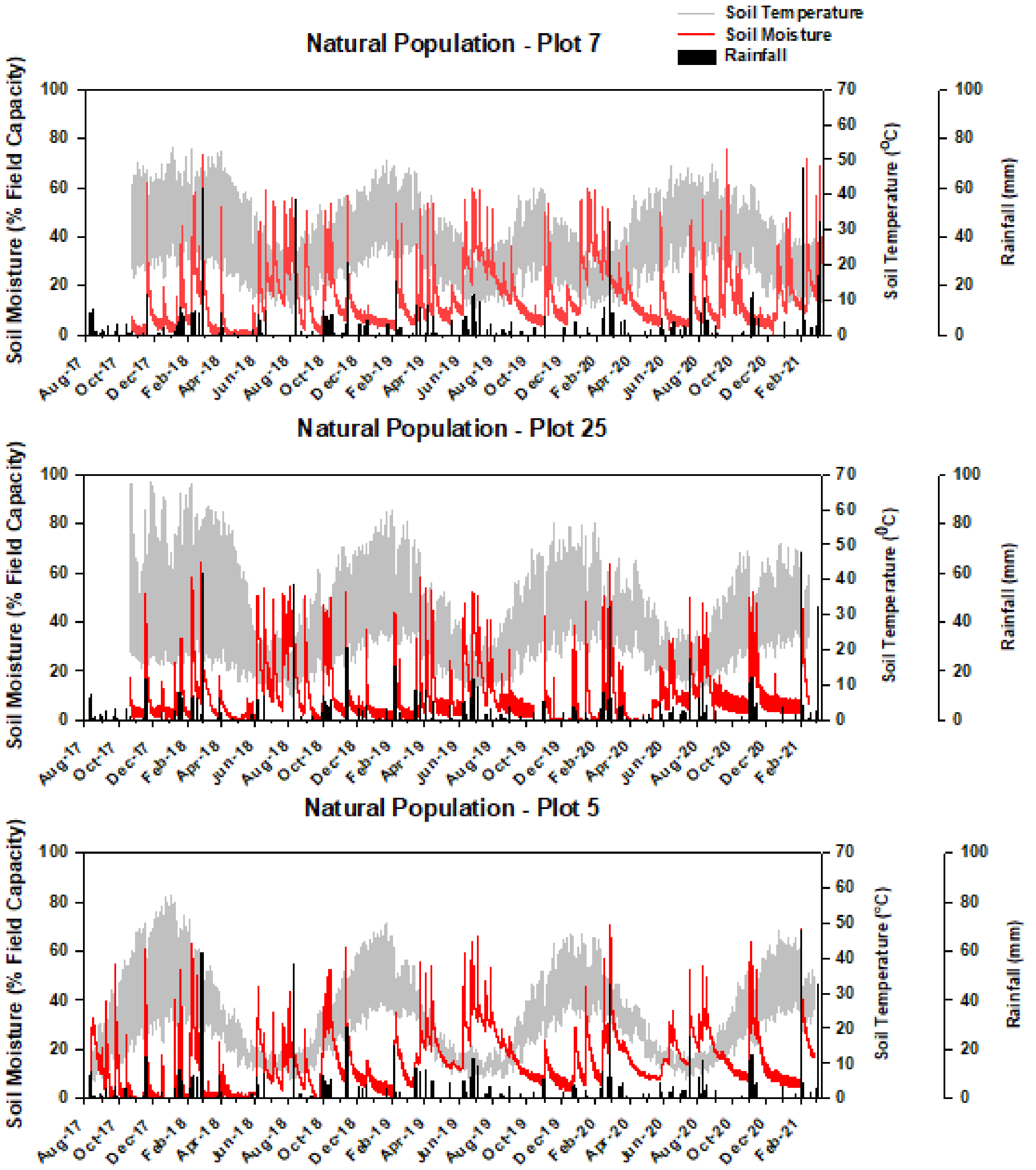


Figure 2.1.3a continued. Soil moisture, soil temperature and total daily rainfall for plots 5, 7 and 25 from August 2017 to February 2021.

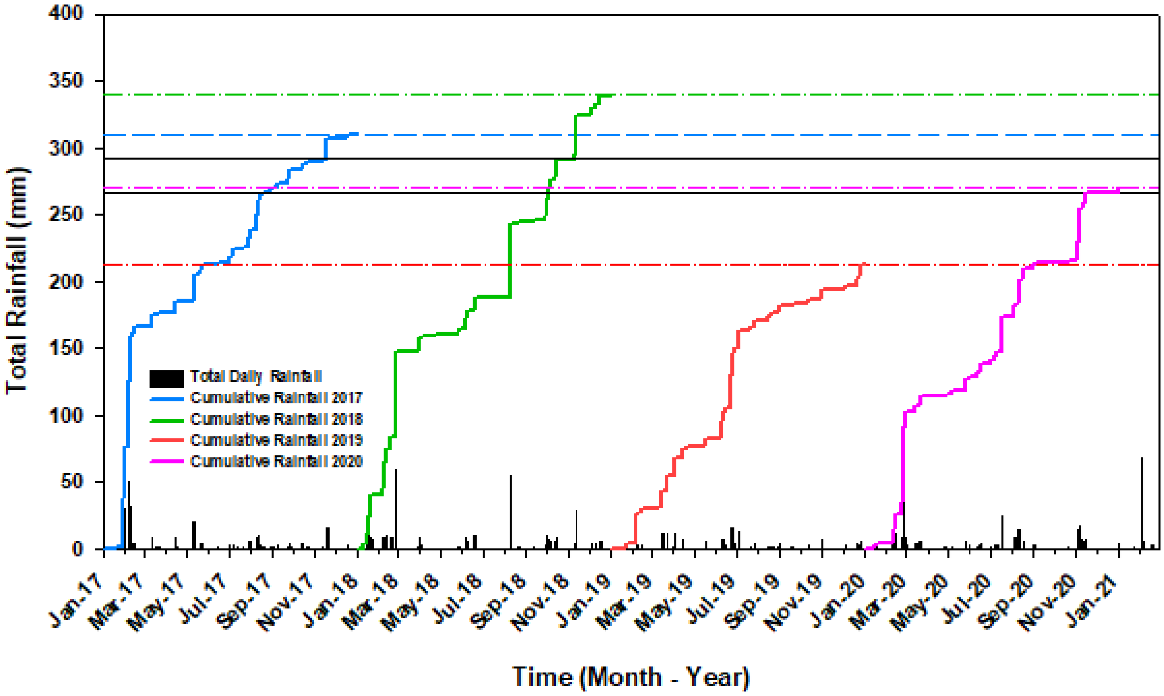


Figure 2.1.3b Cumulative rainfall for 2017 (blue line), 2018 (green line), 2019 (red line) and 2020 (purple line) between January 2017 and February 2021. The dashed horizontal lines indicate total yearly rainfall (2017: dashed blue line; 2018: dashed green line; 2019: dashed red line; and 2020: dashed 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 for Koolyanobbing represented as the black lines.

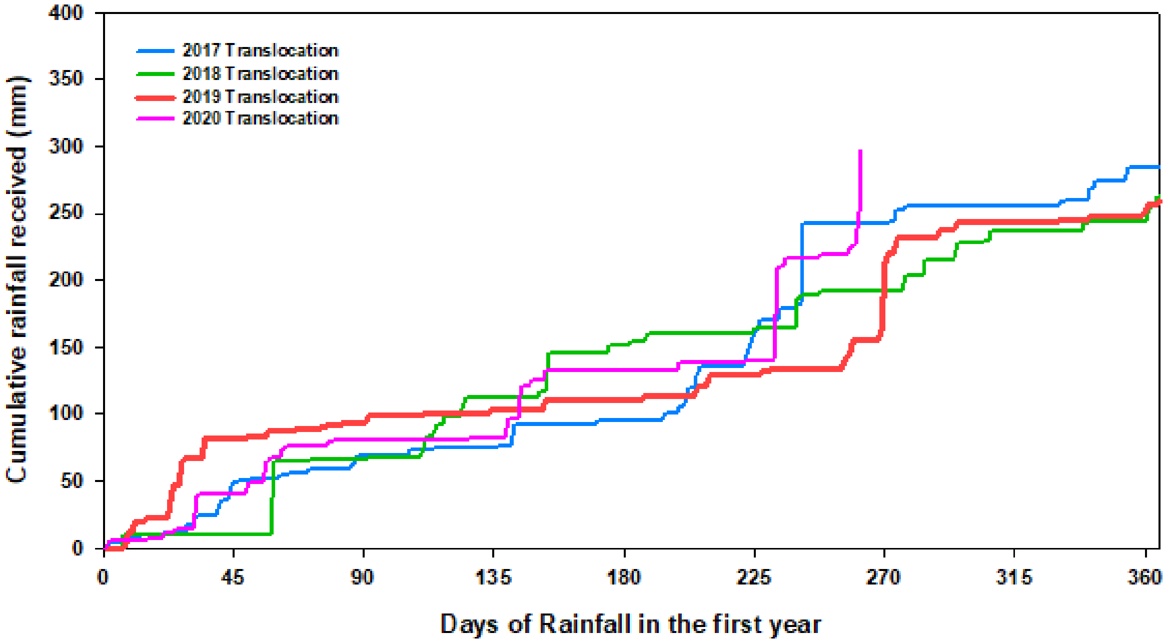


Figure 2.1.3c. Cumulative rainfall within the first year for each translocation trial. The time scale has been standardized to demonstrate how much rainfall the site received after planting for each translocation year (2017-2020). The first 90 days generally coincide with winter conditions, while the last 90 days (270-360 days) were summer conditions.

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.

Current research outcomes:

- Plant responses (i.e. survival) to *in situ* locations were similar among most translocation sites, except one located adjacent to natural populations on the northern side of the ridge where survival was noticeably higher (T23).
- Pre-planting performance of greenstock were similar in height but seedlings had on average one more stem than cuttings.
- Baseline (*ex situ*) plant function responses to drought declined after three days and plants significantly deteriorate after ten days.
- Details of research are in Annual Research Report 3 (Elliott *et al.* 2020).

Greenstock survival was similar among most translocation sites in the initial stages at all three translocation sites (Elliott *et al*. 2018; Elliott *et al*. 2019; Table 2.1.4a), but the survival over multiple summers has showed a varied temporal response among sites. Despite the low survival, the best performing site was an adjacent site (T23) in all four translocation years (See Section 2.2 for further details).

Table 2.1.4a. Summary of greenstock survival for each trial in each translocation site and their spatial location. Table represents cumulative survival of greenstock that are 3.4 years (2017), 2.5 years (2018), 1.6 years (2019) and 8 months (2020) of age as of February 2020. Location was classed according to distance to the natural population (Elliott *et al*. 2018).

Site	Latitude	Longitude	Location class	Distance to population	Greenstock survival			
					2017	2018	2019	2020
T6	-30.87245	119.60269	Outside	<0.1km	0.4%	3.4%	0%	2.5%
T18	-30.88656	119.61919	Outside	0.7km	1.9%	4.5%	0.8%	3.8%
T19	-30.87145	119.60642	Artificial	<0.05km	0%	na	na	na
T21	-30.87394	119.60513	Adjacent	<0.01km	na	1.9%	0%	1.1%
T23	-30.87150	119.60637	Adjacent	<0.02km	4.4%	9.6%	0%	8%
T24	-30.87417	119.61111	Outside	<0.1km	na	3.7%	0%	1.6%

na = not applicable due to no greenstock planted at location

Plants were translocated into five locations with different underlying substrate and context (i.e. distance to nearest *Tetratheca* population; Table 2.1.1a). The pre-planting performance of greenstock was measured to provide a baseline to compare the future growth of translocated plants (average height, stem count).

Growth assessment

The baseline results show that the average height of greenstock was similar between cuttings and seedlings. On average, seedling greenstock had one more stem than cutting greenstock (Figure 2.1.4a). This indicated that seedling greenstock had more aboveground biomass than cutting greenstock of similar age. Both were ~8 months old at planting, which was a similar age to the seedlings planted in the 2019 translocation (greenstock was ~20 months old in the 2019 translocation).

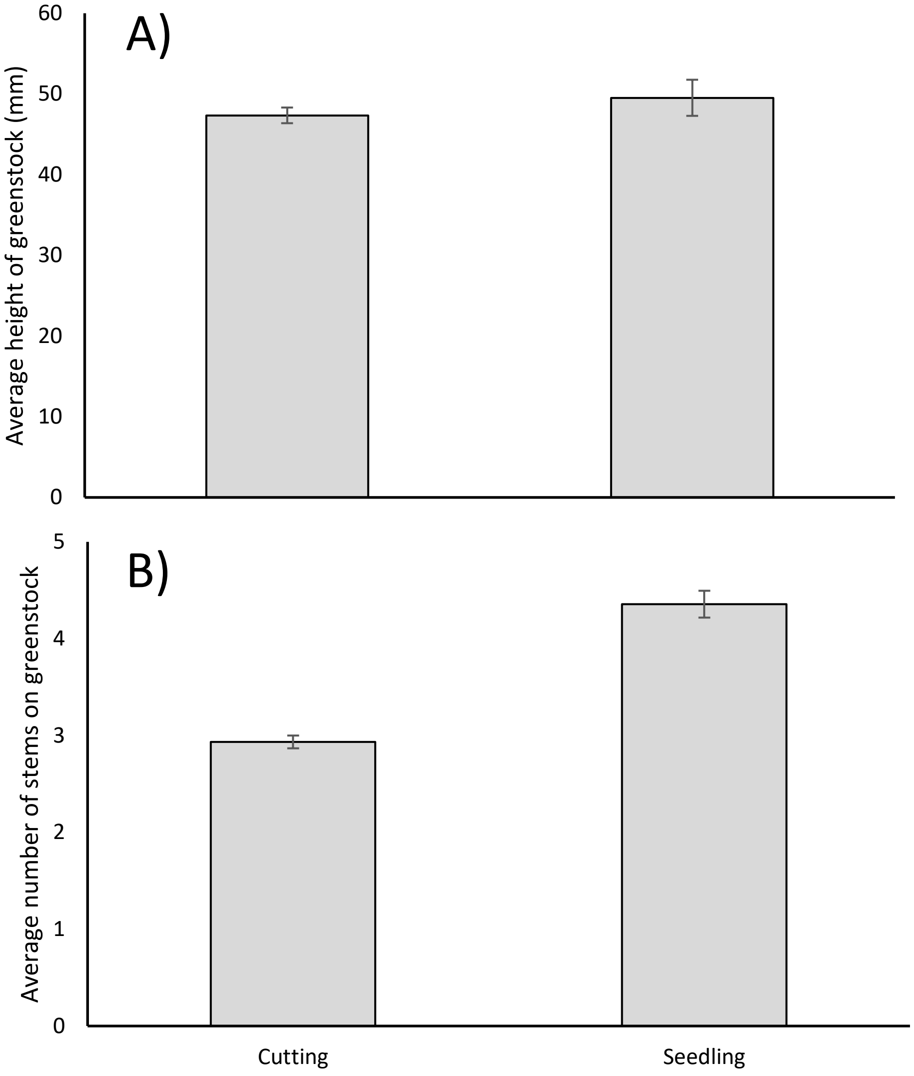


Figure 2.1.4a. Growth performance of greenstock planted in 2020: A) average plant height (mm) and B) average number of live stems per plant. Figure represents mean ± standard error.

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

Current research outcomes:

- Adult plant mortality was recorded (two plants).
- Floral and fruiting phenology differed to previous seasons 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.
- Growth rates in 2020 were <30% and this was lower than 2018 or 2019.
- Natural recruitment (20 seedlings) was lower than 2018 (121 seedlings) or 2019 (252 seedlings) recruitment events.
- Survival of naturally recruited seedlings was only 10% post-summer 2020/2021.

Translocated populations

Current research outcomes:

- 2017 translocation greenstock survival (derived from cuttings) overall was 1.8% after four summers (13 greenstock plants).
- 2018 translocation greenstock survival (derived from cuttings) overall was 4.3% after three summers (46 greenstock plants).
- 2019 translocation greenstock (derived from cuttings and seed) overall was 0.4% after two summers (2 plants).
- 2020 translocation greenstock (derived from cuttings and seed) overall was 3.1% after one summer (22 plants)
- 2020/2021 summer declines of *in situ* greenstock were greater in young greenstock (i.e. 8 months established) than older greenstock (i.e. 42 months established).
- Greenstock survival patterns were similar amongst all translocation sites (within the same year).
- See Section 2.1.1 for current research outcomes for direct seed sowing.

Natural population

In October 2017, mature plants were initially tagged and measured for ongoing reproductive monitoring (plant size, plant health, flower production, fruit production). Table 2.2.1a summarises the number of adult plants tagged for survival, growth and reproductive monitoring and the number that are also being measured for ecophysiology parameters. We recorded no above-ground green foliage for two plants (P9) for 12 and 24 months (Feb 2019 – Feb 2021), and suspect these plants have died. Monitoring should continue to determine if this species can recover after this period of no photosynthetic material, given future rainfall conditions in 2021. This will also assess if below-ground tissues are still viable and capable of regenerating new shoots after this length of time.

Table 2.2.1a. Summary of the number of mature plants in the natural population that were tagged for monitoring in each plot.

Site	General Easting	General Northing	Location on range	No. of plants for demography	No. of plants for ecophysiology
Plot 3	749028	6581554	South	20	4-6
Plot 5	749048	6581507	South	21	4-6
Plot 7	749260	6581695	North	20	4-6
Plot 9	749433	6581518	North	20	-
Plot 10	749531	6581119	South	20	-
Plot 11	749628	6581109	South	20	-
Plot 13	749702	6581074	South	20	-
Plot 16	750011	6580924	North	20	-
Plot 25	749117	6581805	North	20	4-6
Total				**181**	**16-24**

Table 2.2.1b. Summary of set-up and monitoring periods for tagged plants in each plot in the natural population at Koolyanobbing Range.

		Monitoring											
Site	**Set-up**	**1st**	**2nd**	**3rd**	**4th**	**5th**	**6th**	**7th**	**8th**	**9th**	**10th**	**11th**	**12th**
P3	18-25th Oct 2017 Mid Spring	18-21st May 2018	16-23rd Aug 2018	21-28th Oct 2018 1 year	12-20th Feb 2019	7-16th May 2019	22-30th Aug 2019	30-7th Nov 2019 2 years	14-26th Feb 2020	11-20th May 2020	14-21st Aug 2020	23-28th Oct 2020 3 years	22-2nd Mar 2021
P5													
P7													
P9													
P10													
P11													
P13													
P16													
P25													

Floral and fruiting phenology

The average number of floral units (e.g. buds + flowers + fruit) was much lower in 2020 than 2018 or 2019 (Figure 2.2.1a), and corresponds to the pattern of late falling and average rainfall that occurred (Figure 2.2.1j). 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 different between the northern and southern sides; Figure 2.2.1a). However, unlike 2018 or 2019 there was not an increase in floral units in October as expected for spring peak flowering, perhaps due to the late falling average rainfall that may not have started or sustained the flowering season for plants in 2020.

The peak production of buds in 2020 was similar on the northern side of the range and occurred during late winter (Figure 2.2.1b.i) and by mid-spring plants had a combination of buds (e.g. late flowering) or flowers, but mainly had fruits (e.g. early flowering). The mid-spring pattern of reproductive phenology was similar to 2019 patterns, with being lower in quantity and the amount of buds and flowers were similar on both sides of the ridge (Figure 2.2.1b.j). In summary, this data indicates that floral phenology for 2020 was similar between the northern and southern sides of the range, which is different to patterns in 2018 and 2019, perhaps due to a different reproductive response to differing environmental conditions (e.g. temperature, moisture) than previous years.

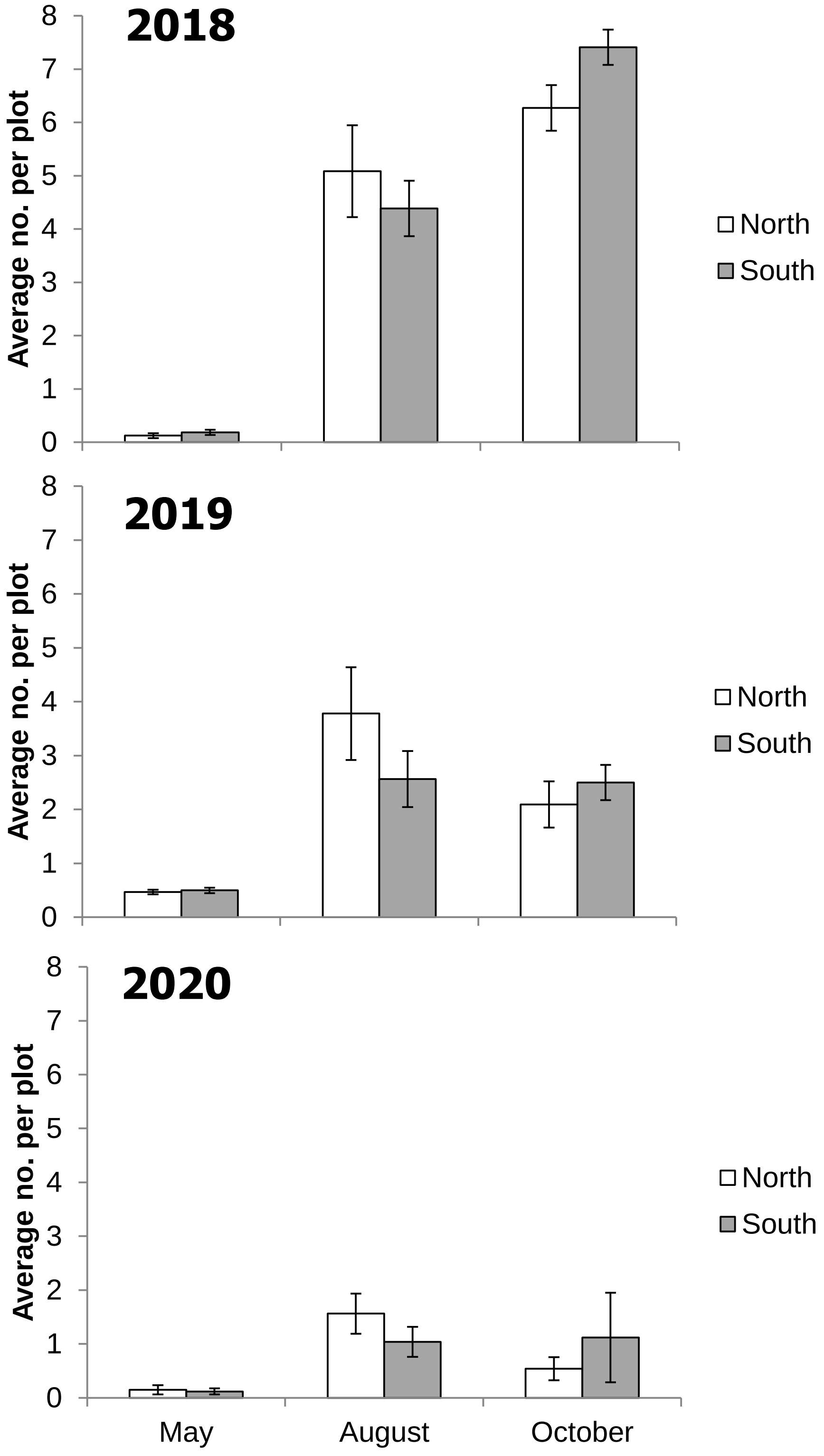


Figure 2.2.1a. Reproductive phenology (mean ± standard error) of plants (*n* = 20 per plot; Table 2.2.1a) in the natural population located on the northern or the southern side of the Koolyanobbing Range. Reproductive phenology represents the average number of floral units (e.g. buds + flowers + fruit) recorded for each month (average number of floral units per branch). Top chart is the 2018 flowering season (Elliott *et al.* 2018); middle chart is the 2019 season (Elliott *et al.* 2020) and the bottom chart is the 2020 flowering season.

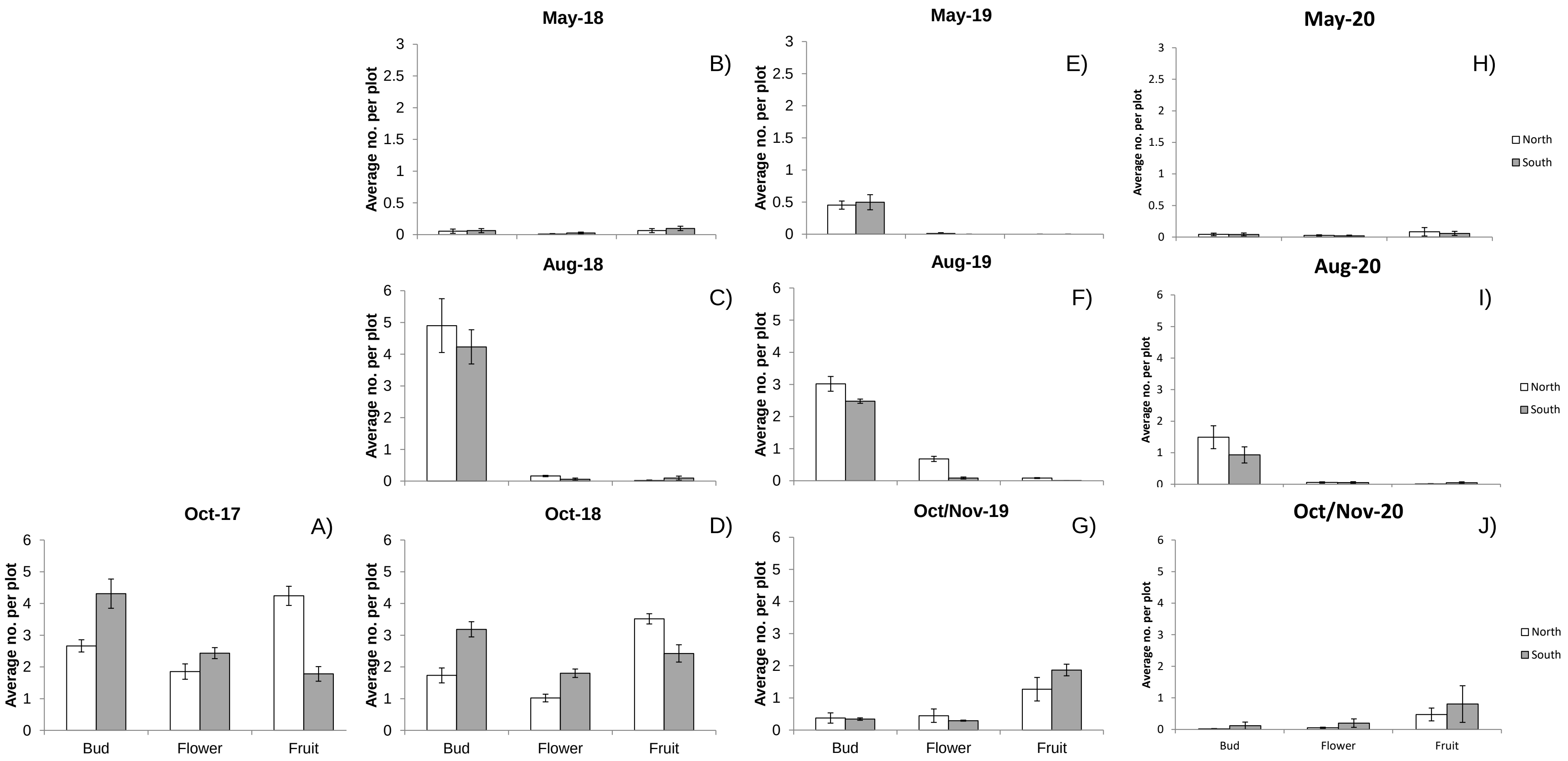


Figure 2.2.1b. Floral and fruiting phenology (mean ± standard error) of plants (*n* = 20 per plot; Table 2.2.1a) located on the northern side (white) or the southern (grey) side of Koolyanobbing Range. The average number of buds, flowers or fruit on plants during A) October 2017; B) May 2018; C) August 2018; D) October 2018; E) May 2019; F) August 2019; G) October/November 2019; H) May 2020; I) August 2020; J) October/November 2020 (average number of floral units per branch).

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). Unlike 2019 patterns, plants on the southern side produced higher amounts of new growth, relative to their size, in comparison to plants on the northern side (Figure 2.2.1c.a). Each plot performed similarly, with a greater proportion of new growth occurring in May before it reached either a plateau or declined in August (Figure 2.2.1c.b). This pattern of reduced growth during winter leading into spring is different to all previous years, except for observations in one plot in 2019 (P9; Elliott *et al.* 2020). This pattern of reduced growth corresponds to a pattern of decreased plant condition observed over the same period (see Figure 3.1.1b). That is, there was an overall decline in plant growth (i.e. number of new stems) of 2-6% between the May and August growing period (Figure 2.2.1c) and a decline in plant condition (i.e. proportion of the plant that is dead) of 6-7% during the same period (Figure 3.1.1b). In addition, the amount of flowering during this period was also lower than previous years (Figure 2.2.1b)

Overall plant growth in 2020 was 22-30% compared to 35-40% in 2019 and 60-70% 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). For example, in 2020, there was late summer rains (above average February) that may have triggered this level of plant growth in late autumn (May; similar response observed in 2018, see Elliott *et al.* 2019). However, below average monthly rainfall during March to June may have limited continuation of this new growth, whereas in previous years, at least one or more of these months received its monthly average. Recovery of plants from a limited or premature growth season will be important to monitor in subsequent seasons.

Natural population recruitment

In August 2020, recruitment of *T. erubescens* seedlings occurred in some of the established monitoring plots in the natural population. These seedlings or existing juveniles were tagged and monitored to establish baseline survivorships of this age cohort in the population. At the time of measurements, 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.

In six of nine monitoring plots (Table 2.2.1a; excluding P7, P25, P9), seedlings were observed in plots, ranging from 1-11 seedlings per plot. In total, 20 seedlings were monitored for growth and survival, which was lower than 2018 or 2019 natural recruitment. Monitoring in February 2021 (post-summer) found that only 10% of these new seedlings had survived (i.e. 2 seedlings). These seedlings emerged and died across a broad range of habitat types, including cliff cracks, rock benches, under adult plants (or not) and deeper soils at the cliff foot-slope. The survival of the 2018 recruits after three summers was 6.6%, and for 2019 recruits was 3.7% after their second summer, both further declines on the previous year.

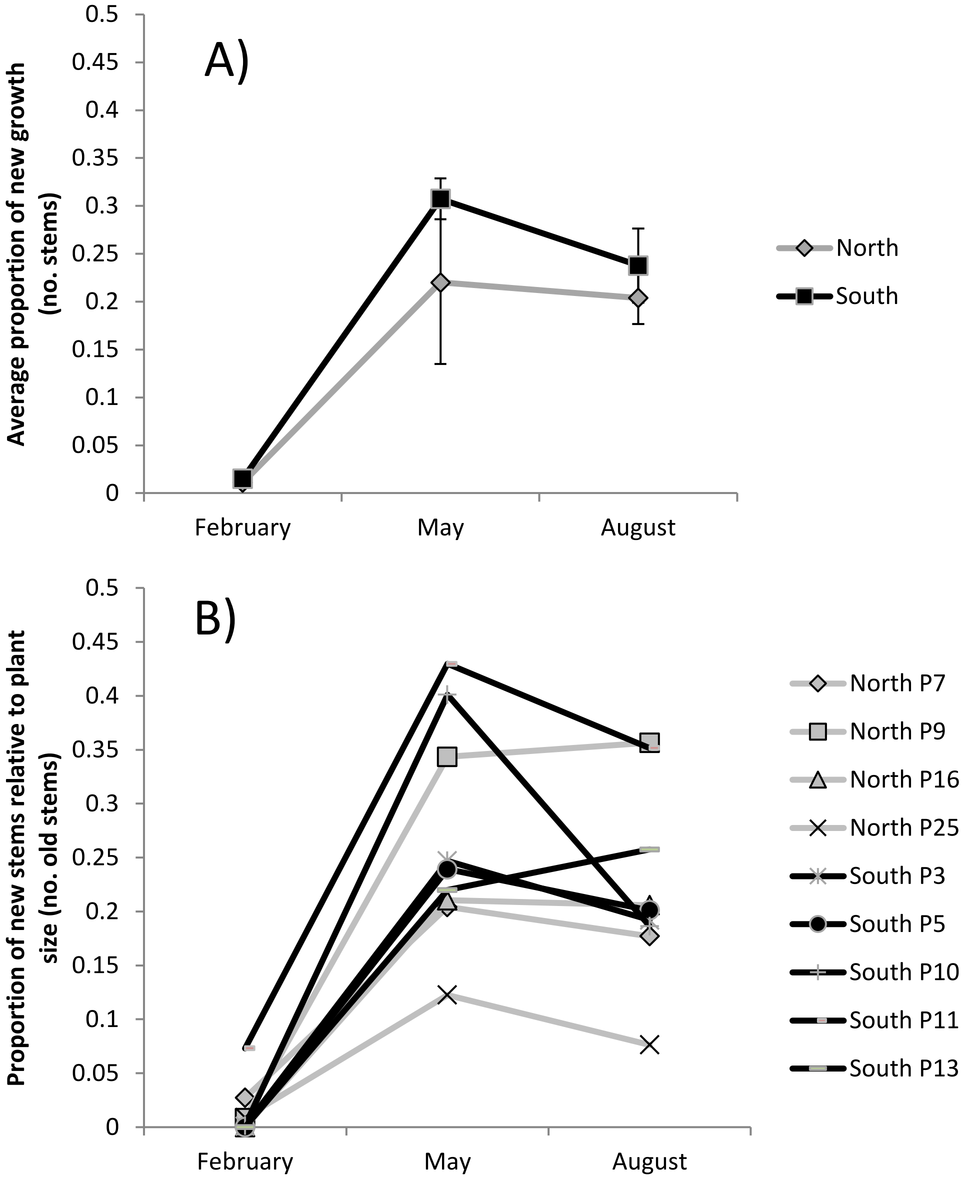


Figure 2.2.1c. Growth of adult plants in the natural population (*n* = 20 plants per plot; Table 2.2.1a), as measured by the number of new stems relative to the number of old stems on each plant over time (February – August 2020). A) overall plant growth (mean ± standard error) for the northern or southern side and B) growth rates (mean ± standard error) within each individual plot, on the northern or southern side.

Translocated populations

See section 2.1.1 for details on translocation site details, including location, characteristics, number of seed sown per translocation and number of greenstock planted per translocation. Details on direct seeding responses and survival is also summarised in this section.

Greenstock planting in 2017 translocation

The survival of greenstock (i.e. at least one cutting per planting unit still alive) declined only in one site after their fourth summer (T18), while the other two held steady (T6 and T23; Figure 2.2.1f). Survival after 3.5 years ranged from 0.4 – 4.4% per site, of the original planting (overall = 1.8% or 13 plants).

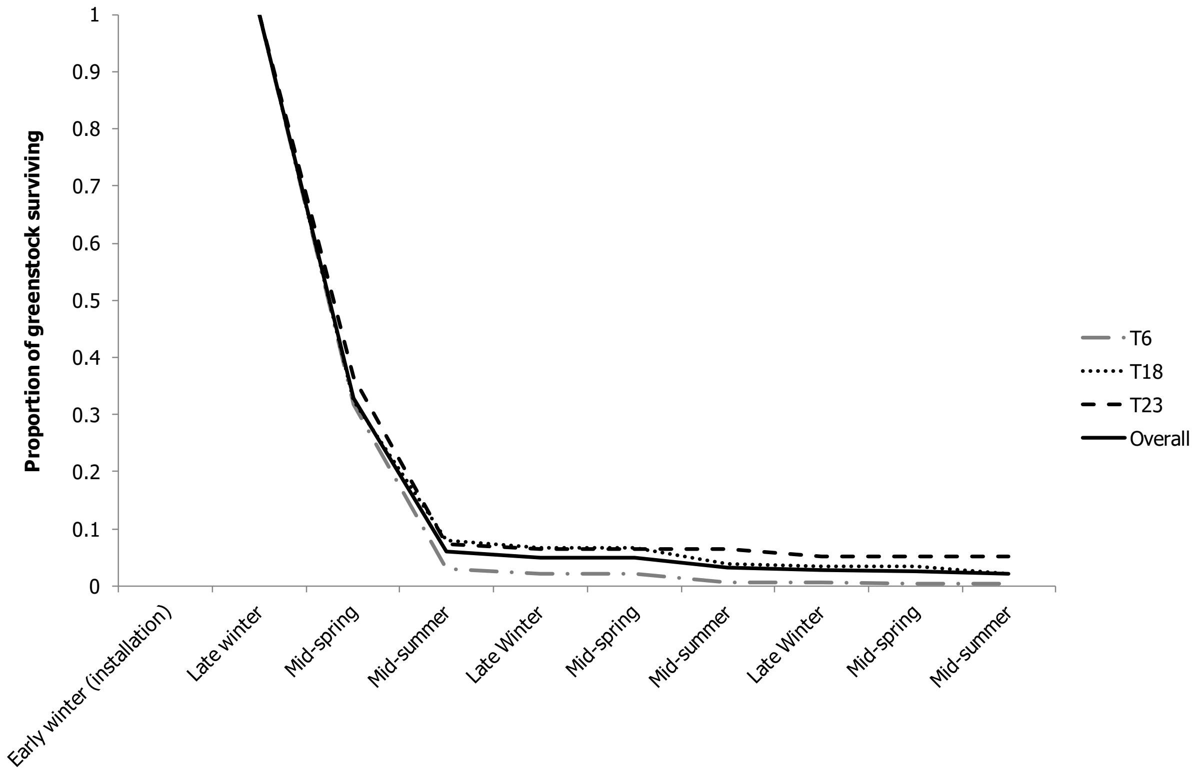


Figure 2.2.1f. Survival of 2017 translocation greenstock from installation (August 2017) to their fourth summer of monitoring (February 2021). Data represents the three translocation sites (T6, T18 and T23) and a combined overall survival rate.

Greenstock planting in 2018 translocation

The survival of greenstock (i.e. at least one cutting per planting unit still alive) declined again after their third summer in two sites (T6 and T23), while the other three held steady (T18, T21 and T24; (Figure 2.2.1g). Survival after 22 months ranged from 1.9 – 9.6% per site, of the original planting (overall = 4.3% or 46 plants).

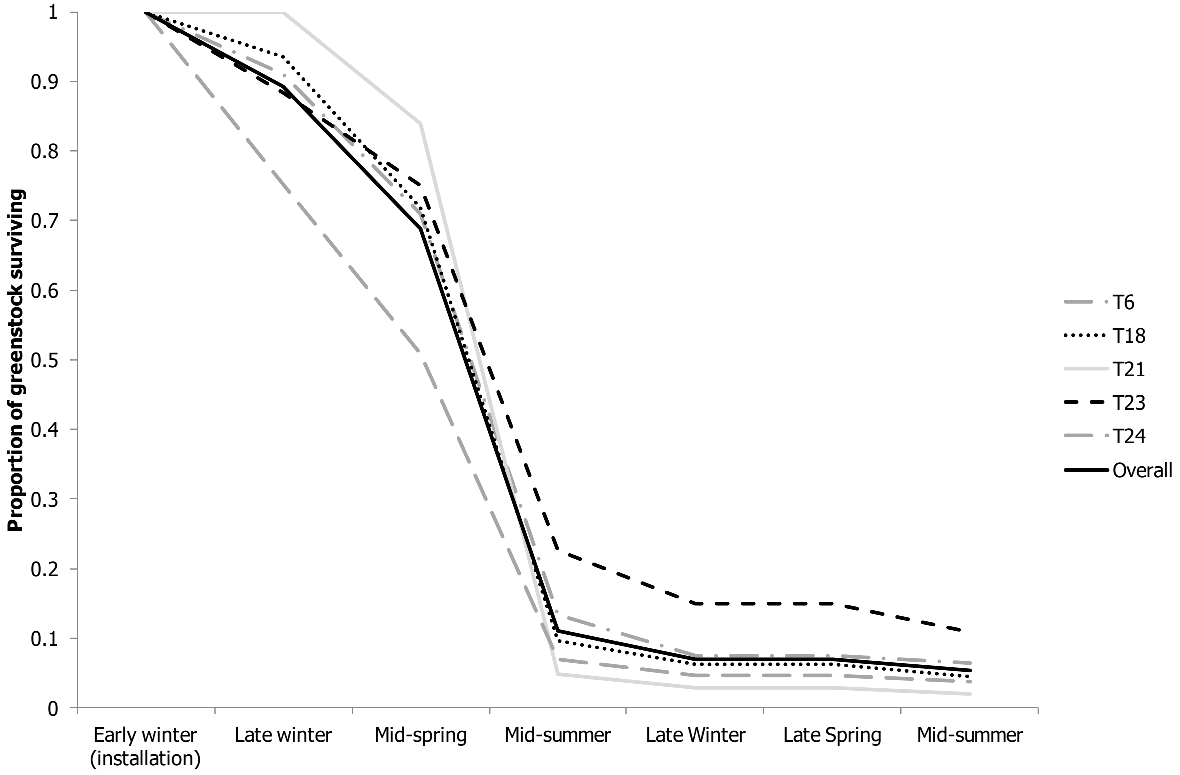


Figure 2.2.1g. Survival of 2018 translocation greenstock from installation (June 2018) to their third summer of monitoring (February 2021). Data represents the five translocation sites (T6, T18, T21, T23 and T24) and a combined overall survival rate.

Greenstock planting in 2019 translocation

The survival of greenstock (i.e. at least one cutting per planting unit still alive) declined significantly by 60% after their second summer (Figure 2.2.1h), with only 2 plants surviving from the previous summer (overall = 0.4% or 2 plants).

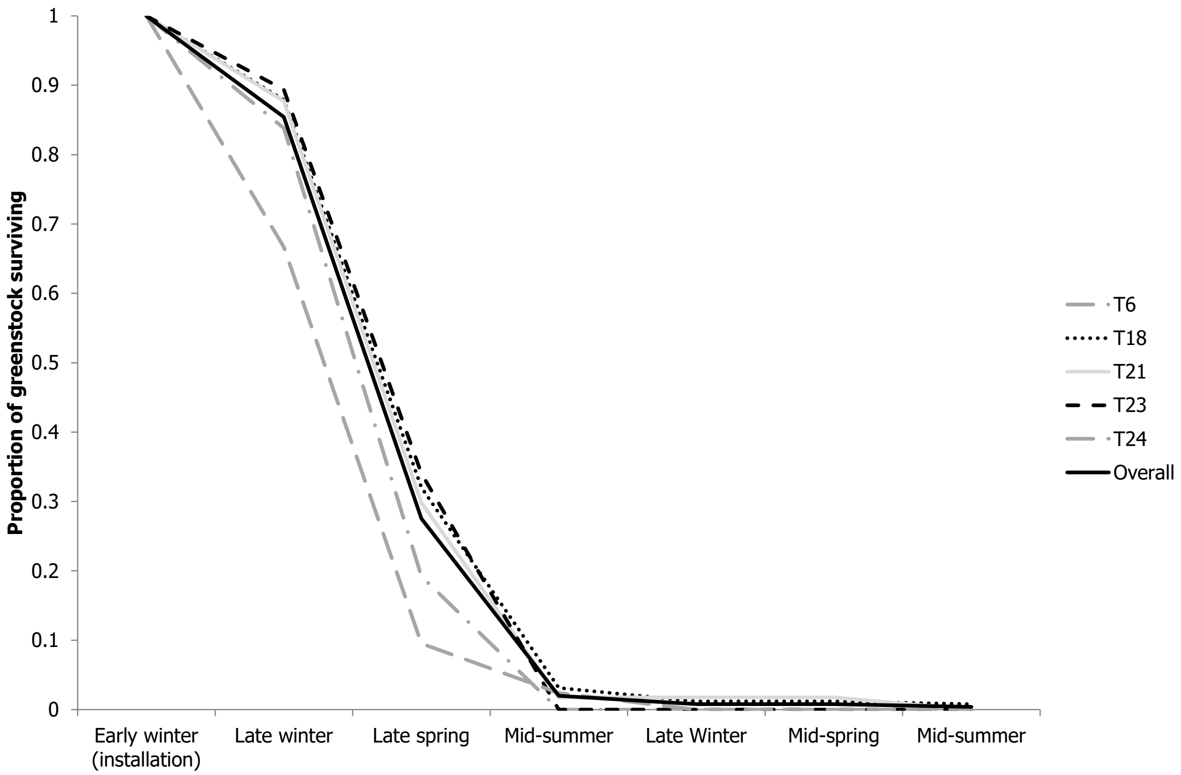


Figure 2.2.1h. Survival of 2019 translocation greenstock from installation (June 2019) to their second summer of monitoring (February 2021). Data represents the five translocation sites (T6, T18, T21, T23 and T24) and a combined overall survival rate.

Greenstock planting in 2020 translocation

Seedlings were germinated and cuttings were collected from stock plants held in the *ex situ* collection at Kings Park in October 2020, propagated and were planted in the five translocation sites in 2020 (Table 2.1.1b; Figure 2.2.1k). Each planting unit had one seedling or 1-4 cuttings as previously outlined for the 2017 translocation (Elliott *et al.* 2018). The treatments tested on the planted greenstock and the number of replicates implemented within each translocation site are summarised in Table 2.1.1h.

The survival of greenstock (i.e. at least one cutting per planting unit still alive) declined significantly after their first summer (Figure 2.2.1i), consistent with the previous 2017-2019 translocation responses. Survival after 8 months ranged from 1.1 – 8.0% per site, of the original planting (overall = 3.1% or 22 plants).

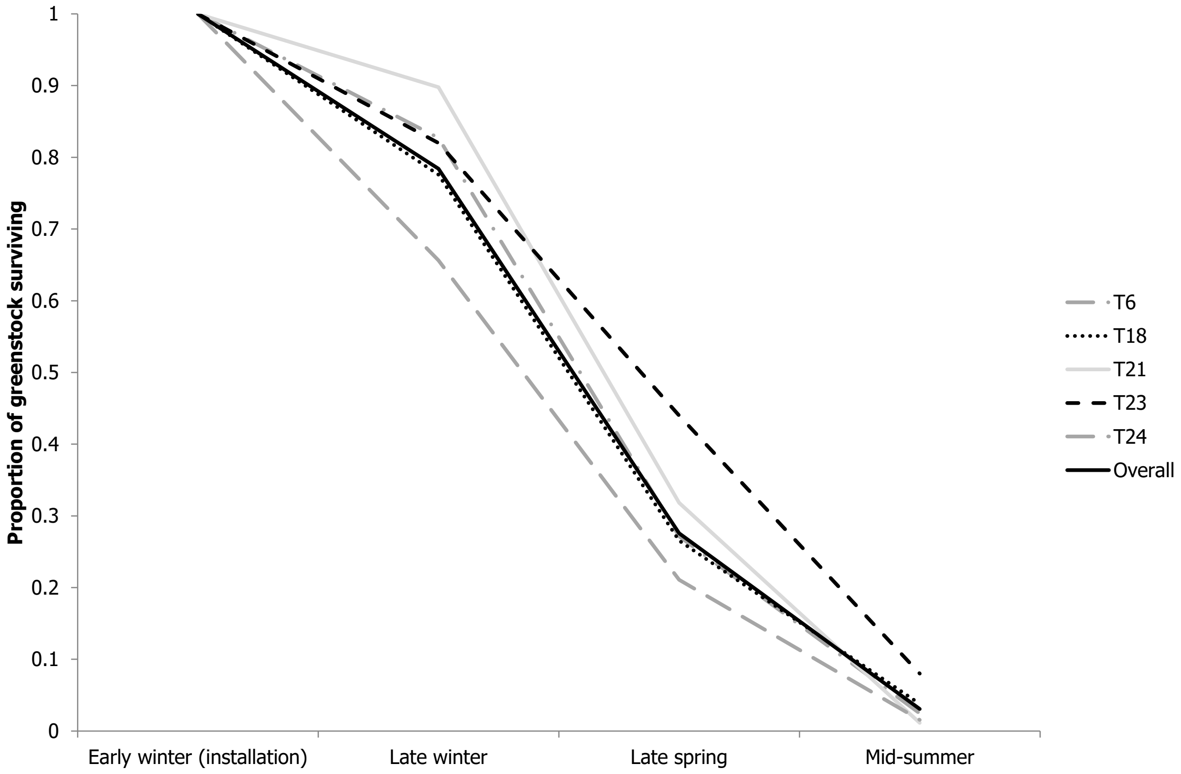
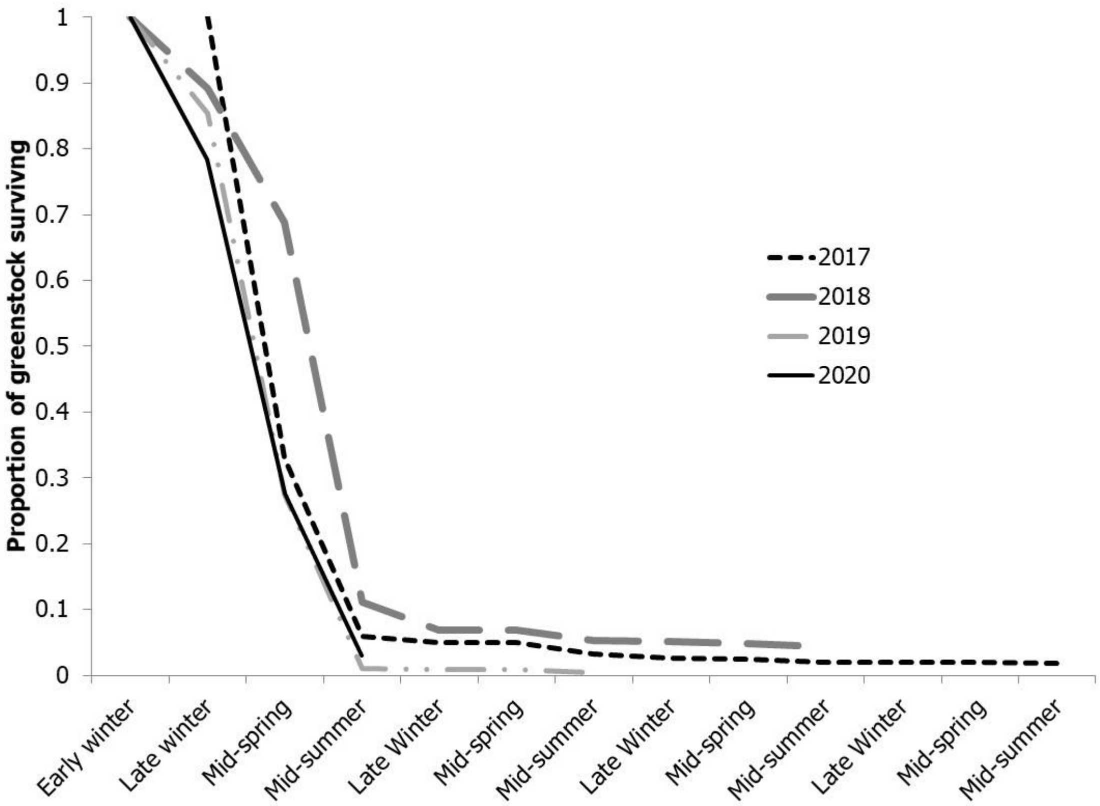


Figure 2.2.1i. Survival of 2020 translocation greenstock from installation (June 2020), late winter (7 weeks; August 2020), mid-spring (18 weeks; October 2020) and after their first summer of monitoring (35 weeks; February 2021). Data represents the five translocation sites (T6, T18, T21, T23 and T24) and a combined overall survival rate.

Year to year comparison

In summary:

- Late winter planting with below average winter rainfall can result in poorer survival, particularly after summer (i.e. 2017 vs 2018).
- 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).
- Below average or average spring rainfall coupled with late summer rains (Feb rain event of >45mm) may have affect greenstock survival. In addition, most rainfall events 6 months post-planting were between 1-10mm (<20 events; >10mm = 3-4 events) that may have also contributed to extremely limited survival (2019 and 2020; Figure 2.1.3c).
- 2019/2020 and 2020/2021 summer declines of greenstock were greater in young greenstock (i.e. planted in 2019 or 2020 and 8 months established) than older greenstock (i.e. planted in 2017 and 42 months established).



29%	29%	85%*	3%	48%	45%	31%	78%	45%*	3%*	5%*	14%*		2017
3%	48%	45%	31%	78%	45%*	3%*	5%*	14%*					2018
31%	78%	45%*	3%*	5%*	14%*								2019
3%*	5%*	14%*								*Late rains			2020

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

Future research:

Natural population and translocated populations

Ongoing development of baseline data on the growth, survival, flowering and seed production of seedlings, juveniles and mature plants in natural and translocated sites will occur to quantify spatiotemporal variation (and any treatment effects). Characterisation of habitat types where these seedlings emerged and survived (or did not) will occur to determine what role it plays in the ongoing survival of seedlings under natural conditions (See Section 2.1.3).

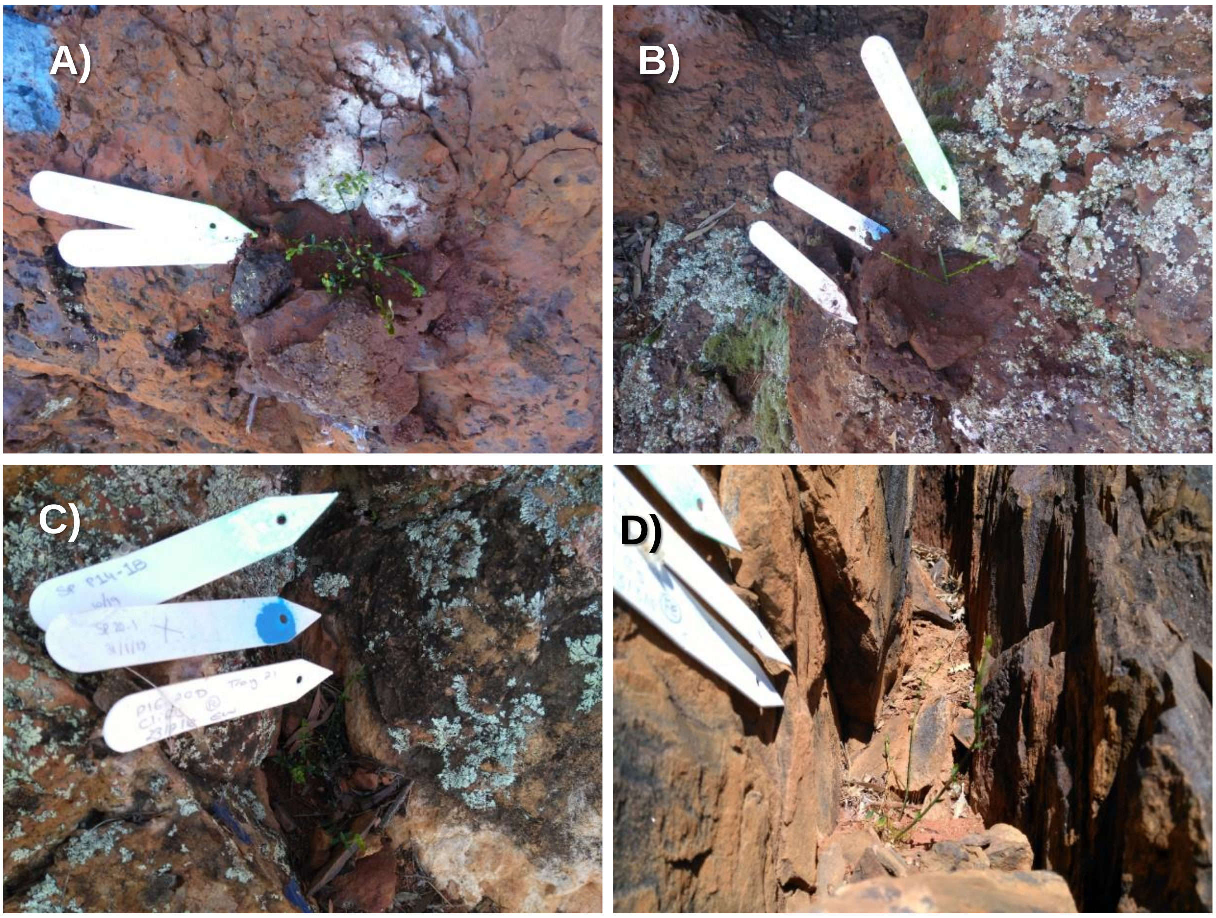


Figure 2.2.1k. Images of the 2020 translocation of greenstock. A) T6 locations of seedling derived greenstock at planting (June); B) T6 location of cutting derived greenstock at planting (June); C) T18 location of seedling derived greenstock in late winter (August); and D) T18 location of cutting derived greenstock in late winter (August). Images A&B C. Elliott and C&D S. Whiteley.

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

Seasonal monitoring will continue across sites and will be paired with environmental data gathered from data loggers (currently at five translocation sites). Kings Park Science will also be using accurate rainfall, wind and temperature data provided by MRL from their weather stations on the top of Koolyanobbing Range. Multiple seasons are required to determine spatiotemporal variation and will be concluded at the end of the project. We anticipate reporting findings following the 2021 data collection.

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

Data are being collected from demographic studies outlined in 2.2 for demographic modelling. This modelling is scheduled for summer 2022.

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

Current research outcomes:

- Survival of translocated greenstock (0.4 - 4.3%) was lower than the survival of monitored adult plants in the natural population (99%; two plants dead in P9).
- The quantity of flowering and seeding of greenstock plants (8-42 months old) was lower than natural adult plants.
- There was no seedling survival from emergents in translocations and low seedling survival from natural recruitment (10% survival; August 2020 – February 2021).

The comparative performance of adult plants in the natural population to the greenstock of the 2017 (3.4 years), 2018 (2.5 years), 2019 (1.6 years) or 2020 (8 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 (see Section 2.1.4 and 2.2.1). Flowering and seed production was quantifiable for oldest translocated plants only. Although not comparable to adults in the natural population (i.e. size, maturity etc.), greenstock plants produced 1-33 flowers per greenstock plant (of those that flowered). Developing fruits were observed, but were immature at the time and could not be collected and the plants too fragile to place organza bags on them. Maturity and ongoing survival of greenstock plants will ensure comparative performance measures can occur in future seasons. Emergence of seedlings from direct seeding lines in the translocations (see Section 2.1.1) or the natural population (see Section 2.2.1) was poorer in 2020 than previous years and their survival (limited) was only detected in the natural population (10%) after the 2020/2021 summer.

Observations of an undefined type of disturbance (e.g. possibly herbivory) were noted, as fresh mammal scats were observed at translocation sites and there was a large increase in the number of greenstock plants that had no aboveground biomass (alive or dead) to measure, in comparison to previous years. It is suggested that cameras are placed at translocation sites post-planting and over the first summer to confirm and identify the type disturbance.

Future research:

Ongoing comparative performance between plants in natural and translocated sites will occur to quantify spatiotemporal variation for natural adult plants across seasons, greenstock across seasons, as greenstock matures in translocations and comparisons between both groups (and any treatment effects). Ongoing monitoring of plants impacted by disturbance events is necessary to determine survival/recovery outcomes and/or initiate management actions to ensure survival.

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.

Current research outcomes:

- Ecophysiological performance was similar between juvenile and adult plants in the natural population.
- Plant condition of those in the natural population, as measured by the proportion of a plant that had recently died, was at its lowest during the peak growing season (May-Aug) and highest during summer (Feb). This has been consistent across years (2018-2020).
- Relative plant condition changed between February 2020 and February 2021, with a 19.7-25.8% decline in plant condition (i.e. greater proportion of the plant had died) in 2021 over its relative condition in 2020.
- Each plot responded similarly to 2020 conditions, with increases in the proportion of a plant that was dead from May through to February 2021, indicating declines in 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 late winter (August 2020) and mid spring (October 2020) 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 2018 and 2019 (Elliott *et al.* 2019; Elliott *et al.* 2020).

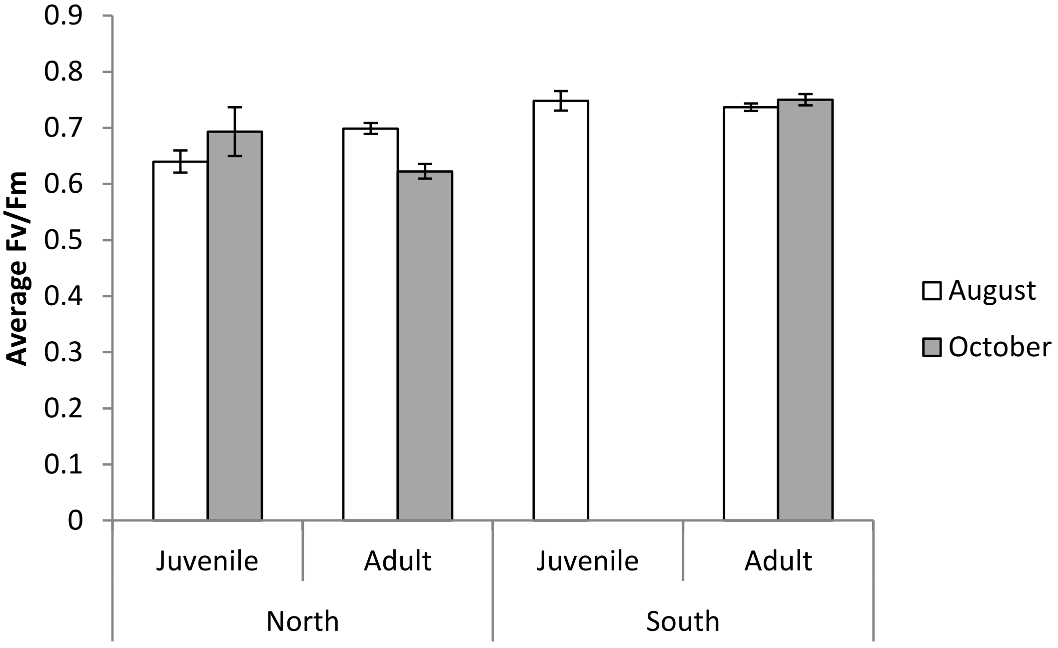


Figure 3.1.1a. Average Fv/Fm measurements of adult plants (*n* = 26-61 plants) and juveniles (*n* = 2-6 plants) in the natural population during two periods in 2020.

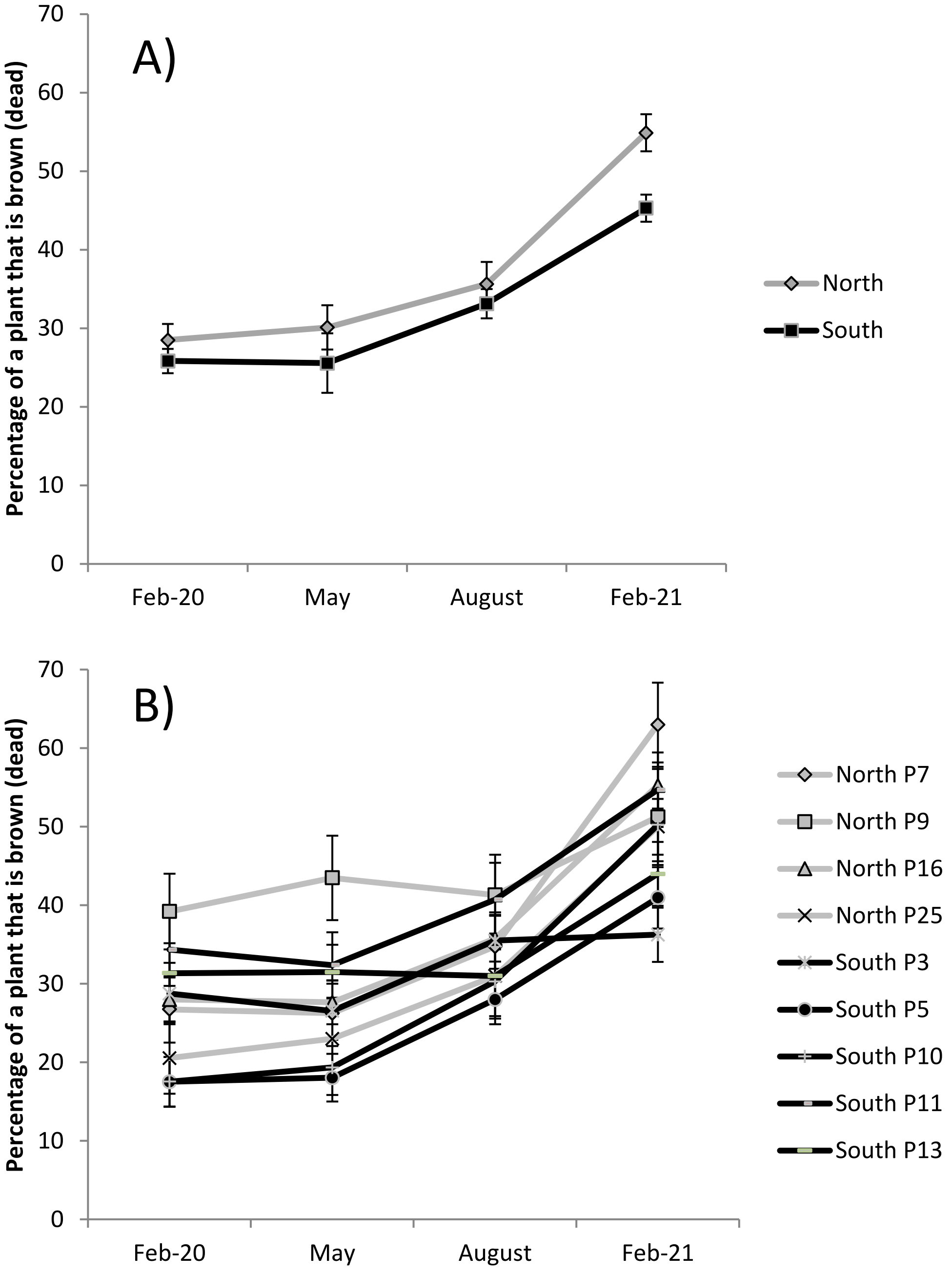


Figure 3.1.1b. Condition (mean ± standard error) of adult plants (*n* = 20 per plot; Table 2.2.1a) in the natural population, as measured by the proportion of an adult plant that is brown (i.e. newly dead plant tissue less than six months is a rich brown, not faded grey/white) over this time period (February 2019 – February 2020). 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.

The peak condition of monitored plants (i.e. majority of the plant was green) occurred mainly during May in 2018 (see Elliott *et al*. 2019), but in 2019 there was no apparent peak overall, as plant condition showed a steady and stable decrease in health from summer to summer (see Elliott *et al*. 2020). In 2020, there was also no apparent peak in plant condition, despite plants putting on new stems (see Figure 2.2.1c). In 2020, there was a steady decline in relative plant condition (i.e. proportion of the plant that was brown) from summer, through the growing season (May-Aug) and into the following summer. This pattern was similar for the northern and southern sides of the ridge (Figure 3.1.1b.a). Unlike the pattern in 2019, each individual plot had a similar response to the 2020 conditions (Figure 3.1.1b.b).

Plant condition of those monitored in the natural population, as measured by the proportion of the plant biomass that had recently died (% brown), had changed when comparing February 2020 with February 2021 (Figure 3.1.1b.b). There was an overall decline in plant condition in summer 2021, where a greater proportion of the plant had died that was 19.7-25.8% over and above their relative condition at the same time the previous year (2020). 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. Such a change in relative plant condition between years has not been previously observed and is cause for concern.

Previous trials assessing the drought responses under glasshouse conditions (see Section 2.1.4 in Elliott *et al.* 2020) indicated that plant health declined significantly after prolonged periods of low soil water potentials (-0.91 to -1.34 MPa). These soil water potentials occurred after withholding water from potted plants for 13-14 days. There is a possibility that the plants have reached this threshold that has caused reduced plant condition, but further modelling would need to investigate the soil moisture dynamics on performance windows (both condition and function) based on plant-water interactions. Controlled drought trials would inform against the patterns observed in the field and should be considered for identifying optimal watering treatments in any future translocation planning.

Future research:
To understand plant function at different ages, plants will need to be propagated from seed and grown to seedling, juvenile and adult plant stages, as these life stages may represent different tolerances to stress. The source material can be generated from seed experiment methods outlined in Program 1. Germinated seeds should be propagated into large pots and grown to different stages over two years. At each growth stage, a subset of plants (8-16 samples/treatment) should be used to understand drought-response, gas exchange (photosynthesis, transpiration and stomatal conductance) and chlorophyll fluorescence. Plant function and condition of those tagged in the natural population should continue to be monitored to determine the magnitude of variation in their responses to seasonal changes.

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

Current research outcomes:

- Stomatal conductance varied among seasons and between aspects, most likely driven by soil water availability and temperature/site exposure.

- Summer measurements for 2017 and 2018 were on average the lowest for all species, which coincided with elevated leaf temperatures and a decrease in chlorophyll fluorescence.
- Summer measurements for stomatal conductance in 2020 and 2021 were elevated due to measurements coinciding with rainfall events.
- The measurements in spring in 2020 coincided with increased leaf temperatures and decreased stomatal conductance, which were a result of the decreased number of rainfall events in spring.
- *Tetratheca erubescens* plants on NE-facing aspects have consistently lower performance during autumn, winter and spring, but slightly elevated responses during summer than plants on SW-facing aspects.
- Leaf temperatures were higher in NE-facing sites indicating hotter site temperatures.
- Chlorophyll fluorescence declined between spring and summer periods and recovered between autumn and winter periods. Further corroborating stomatal conductance and leaf temperature measurements, NE-facing sites are generally showing lower Fv/Fm ratios suggesting an increased stress risk to environmental conditions.

Methods and Results

Previous ecophysiological assessments were quantified using a LI-COR 6400XT gas exchange system. While providing high quality data, measurements were constrained by the mobility of device, accessibility to plants on rocky locations and measurement durations. As an alternative to the LI-COR 6400XT, porometer measurements (SC-1 Leaf Porometer, Decagon Devices Inc. Pullman) were conducted to increase the sample size and the spatial resolution of plants measured across sites. While only providing measurements for stomatal conductance and leaf temperature, the porometer is more mobile across the landscape and measurements are more rapid, thus increasing the sample size. Measurements were conducted in four natural *T. erubescens* populations (NE Plots: 7 and 25; SW Plots: 3 and 5) in spring, summer, autumn and winter seasons in 2018-2020, and the summer of 2021 on 8-12 adult *T. erubescens* plants per plot (*n* =16-24 plants per NE and SW aspect) on green new/fully developed phyllodes. All measurements were conducted in the morning between 0700-1130am. Leaf temperature was quantified simultaneously with stomatal conductance measurements to determine how effective plants were regulating their stomata relative to the environment. Chlorophyll fluorescence was measured after dark adapting the same leaf for 10 minutes. These measurements were conducted on 4-6 *Banksia arborea* and *Eremophila decipiens* plants per plot (*n* = 8-12 plants per NE and SW aspect) that co-occur with *T. erubescens*, to quantify environmental variation on a species-level across natural populations. In total, thirteen measurement blocks have been completed since summer, 2017.

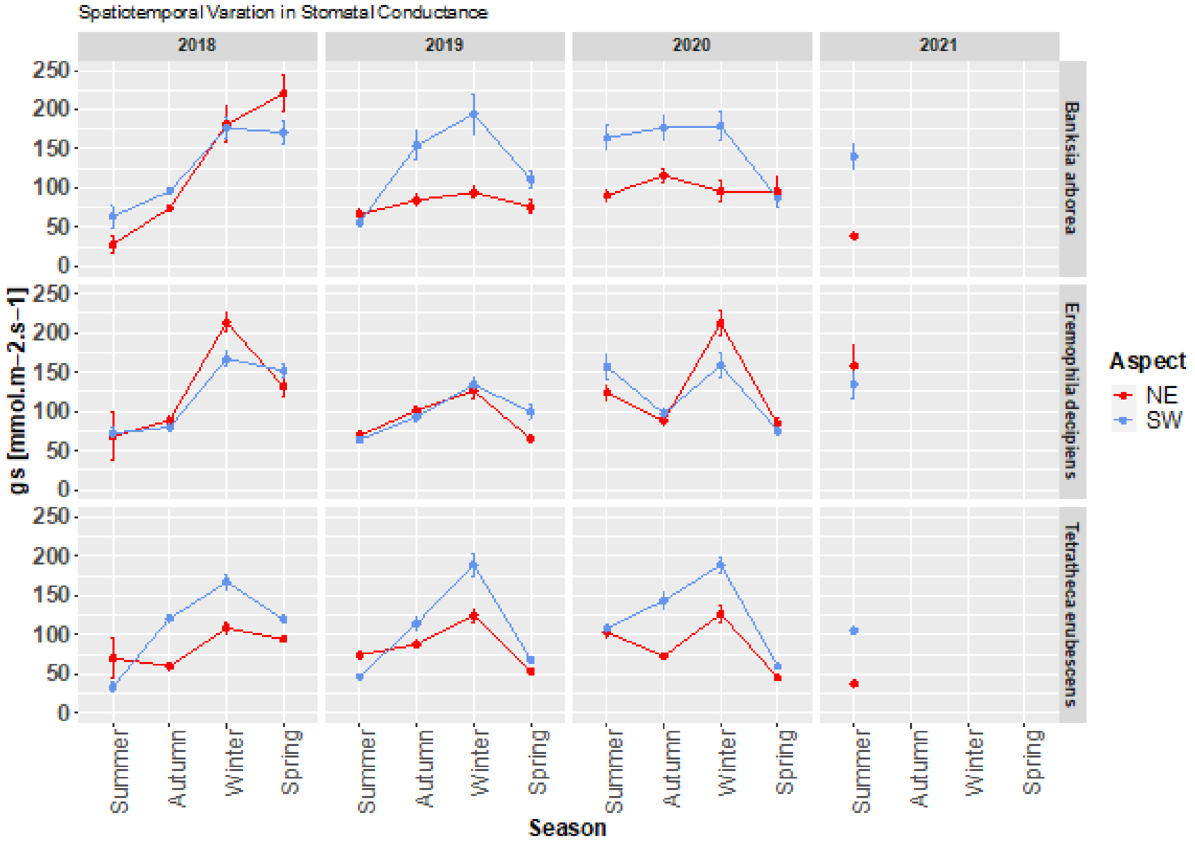


Figure 3.1.2a. Stomatal conductance (mean ± standard error) of *Tetratheca erubescens* (*n* = 16-24) and two common BIF species (*Banksia arborea* and *Eremophila decipiens*; *n* = 8-12) in NE-facing sites (P7 and P25) and SW-facing sites (P3 and P5).

There was seasonal variation in stomatal conductance between NE- (P7 and P25) and SW-facing (P3 and P5) sites (Figure 3.1.2a). As demonstrated by winter measurements that were always characterised by highest plant performance, and summer characterised by lowest plant performance (with exception to the summer period of 2020 and 2021 as measurements coincided with rainfall events occurring prior to the measurement window). For T. *erubescens,* there was consistently lower stomatal conductance in NE-facing sites compared to SW-facing sites. Compared to the other two species, measurements in 2019 for *T. erubescens* were consistent with measurements from 2018. Both *Banksia arborea* and *Eremophila decipiens* demonstrated higher performance values in 2018 compared to 2019. This response is likely a result of lower rainfall observed in 2019 (see Figure 2.1.3b). For *T. erubescens,* despite the SW-facing sites having higher stomatal conductance responses across autumn, winter and spring seasons, both summer 2018 and 2019 measurement points were lower than NE-facing sites. The summer measurements in 2020 and 2021 on average demonstrated much higher responses compared to previous summers on SW-sites, due to greater plant available water in the BIF substrate following rainfall during the measurement period. The NE-sites demonstrated the lowest performance for stomatal conductance. For all species there were increases in stomatal conductance compared to the previous spring measurement.

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 matched by the leaf temperatures in the recent 2021 summer measurements. Taken together, these values indicate that measured plants were experiencing a prolonged period of heat across the landscape.

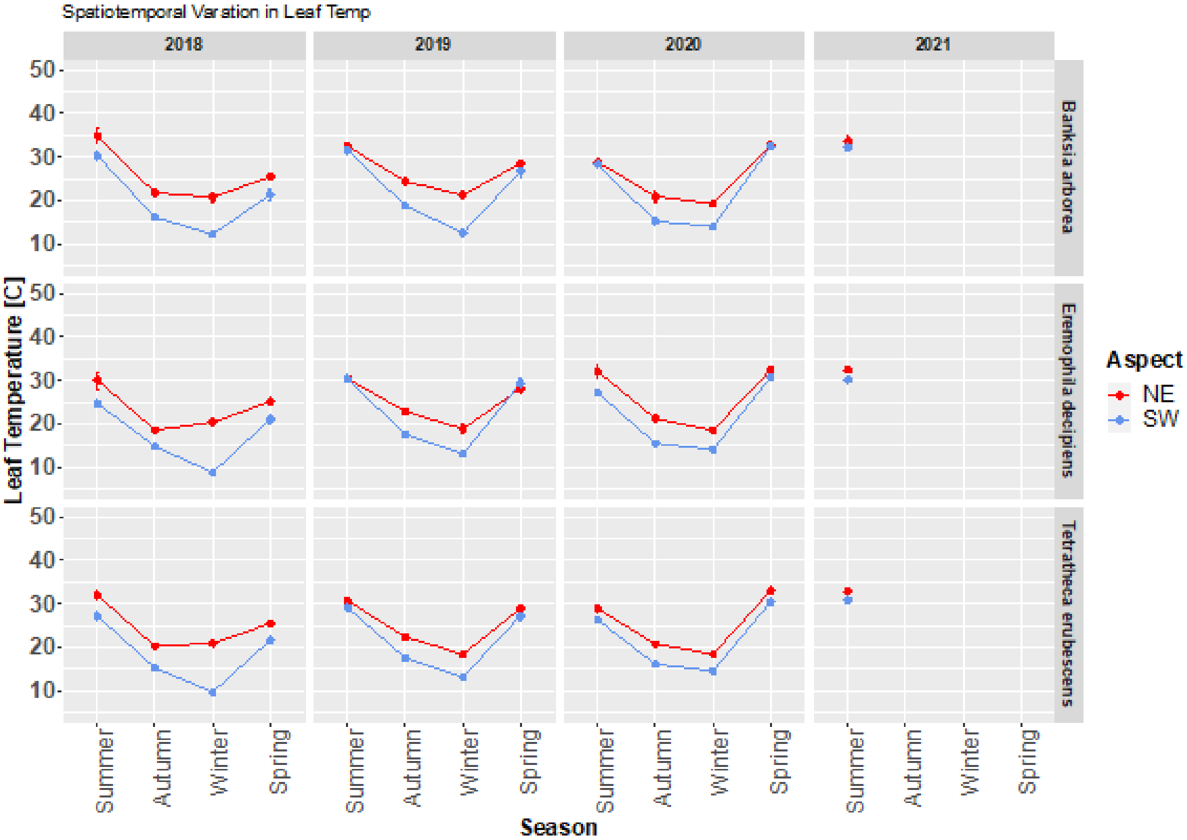


Figure 3.1.2b. Leaf temperatures (mean ± standard error) of *Tetratheca erubescens* (*n* = 16-24) and two common BIF species (*Banksia arborea* and *Eremophila decipiens*; *n* = 8-12) in NE-facing sites (P7 and P25) and SW-facing (P3 and P5) sites.

Chlorophyll fluorescence generally declined between spring and summer periods, with recovery observed between autumn and winter. For all species, lower chlorophyll fluorescence coincided with higher leaf temperatures in NE-facing plots (Figure 3.1.2c). Further corroborating the low stomatal conductance and high leaf temperatures measures 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.

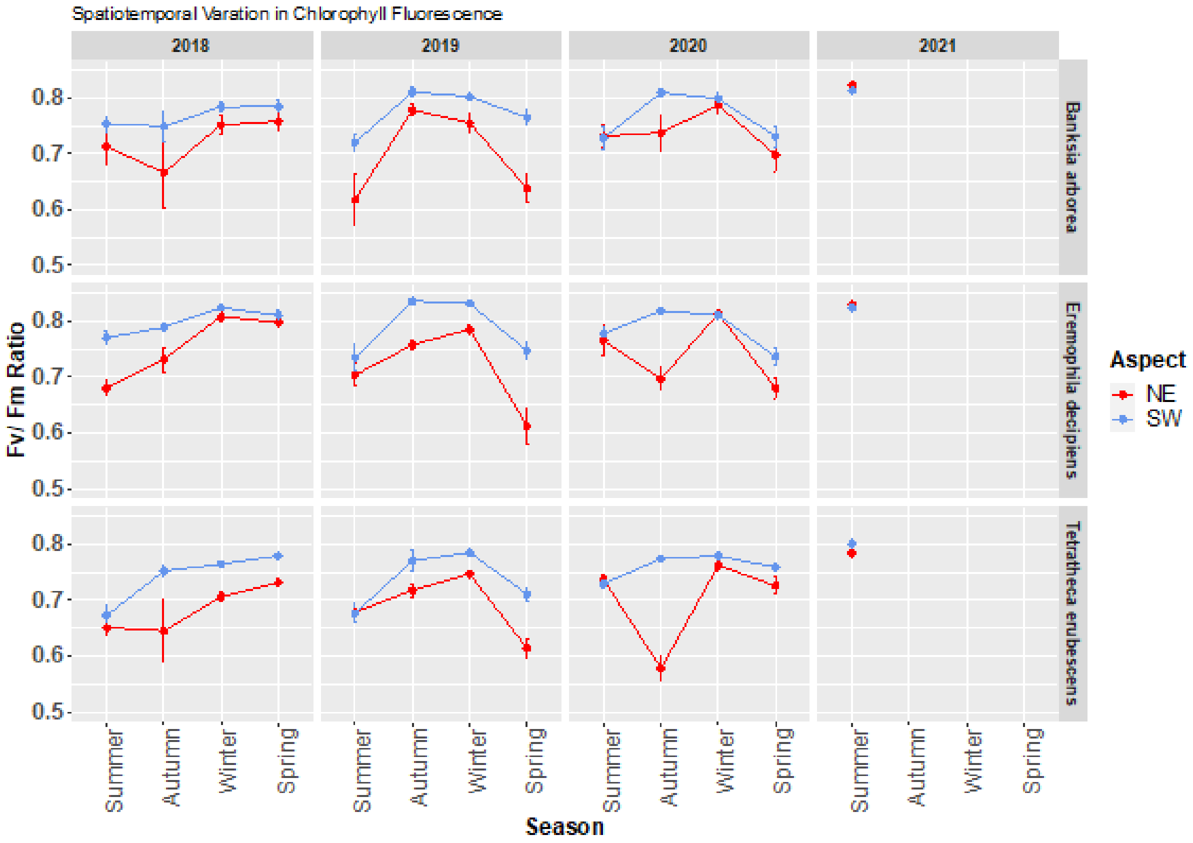


Figure 3.1.2c. Chlorophyll fluorescence (mean ± standard error) of *Tetratheca erubescens* (*n* = 16-24) and two common BIF species (*Banksia arborea* and *Eremophila decipiens*; *n* = 8-12) in NE-facing sites (P7 and P25) and SW-facing (P3 and P5) sites.

Applications

- The data demonstrate seasonal changes in plant performance between summer and winter periods – these conform to the active plant growth and senescence cycles (reported in Section 2.2).
- The common species, *B. arborea* and *E. decipiens*, demonstrate similar patterns, with reduced plant performance observed during summer. The ridge aspect showed strongest variation, with NE-facing sites generally more exposed to direct sun-light and thus hotter leaf temperatures, lower stomatal conductance and chlorophyll fluorescence compared to the shaded SW-facing sites.
- In summary, plants (*T. erubescens, B. arborea* and *E. decipiens*) growing in NE-facing sites appeared functionally more stressed. This may have implications for future translocation designs, as NE-facing sites may expose cuttings to greater environmental stress.
- The rainfall that coincided with the measurements in summer 2020 and 2021 increased soil water availability, which increased plant performance in the SW-facing aspect, but not always in the NE-facing aspect (compare 2020 summer, with 2021 summer measurements for stomatal conductance).

Future research:
Ongoing measurements will occur over the next year to quantify spatiotemporal variation for plants of *Tetratheca erubescens* (and the two common species) to confirm that plant function is responding differently than other more common species in the same habitat, during the same season.

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

Current research outcomes:

- Rock strata contain potential pockets of accessible moisture for roots (as outlined in Elliott et al 2019).

Ongoing analysis of the materials collected for this objective is required before an identification of the ecophysiological strategies employed by plants on banded ironstone ranges can be reasonably made. Additional sampling may be required to improve the resolution of the analysis, should the opportunity occur to collect more samples.

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

Investigations in Sections 3.1.1 and 3.1.2 are currently in progress and will contribute towards underpinning variation in plant function. Kings Park Science will report findings following the 2021 data collection. Current investigations for environmental factors include leaf temperature, ambient temperature, soil temperature, evaporation, rainfall and aspect.

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

Current research outcomes:

- There is variation in plant performance between natural and translocation sites, and populations in planted facing northeast and southwest aspects.
- As cuttings establish over time, they perform similarly to plants in natural reference sites.
- Lowest performance indicators are measured from more recent translocation trials, as populations are experiencing highest mortality rates.

Natural vs translocation performance
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. 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 (Figure 3.1.5a) and lower leaf temperatures (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; Figures 3.1.5a,b) and increased mortality rates in spring 2019 and summer 2019/20.

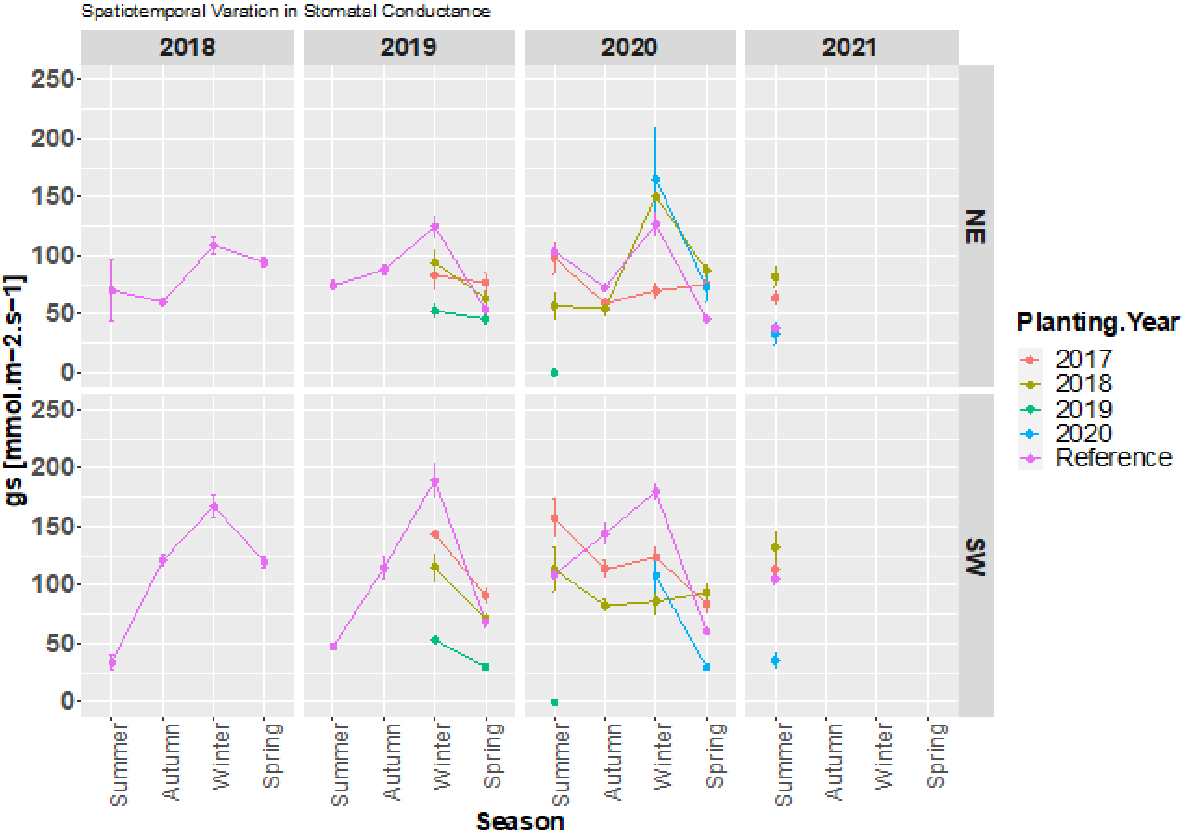


Figure 3.1.5a. Stomatal conductance (mean ± standard error) of *Tetratheca erubescens* in natural/ reference sites (NE-aspect plots: P7 and P25; SW-aspect plots: P3 and P5), and cuttings that were planted in translocation sites (NE-aspect: T23; SW-aspect: T6. The translocation sites contained cuttings that were planted in 2017, 2018, 2019 and 2020 Ecophysiological assessments in translocation sites commenced in the winter season of 2019 for cuttings that were planted in 2017-2019, and assessments commenced in the winter season of 2020 for the 2020 translocations.

There were elevated performance values from the 2017 cuttings in the SW-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 functioning 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 (Figure 3.1.5a). In the SW-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 functioning, with lowest performance measured in the 2021 summer period.

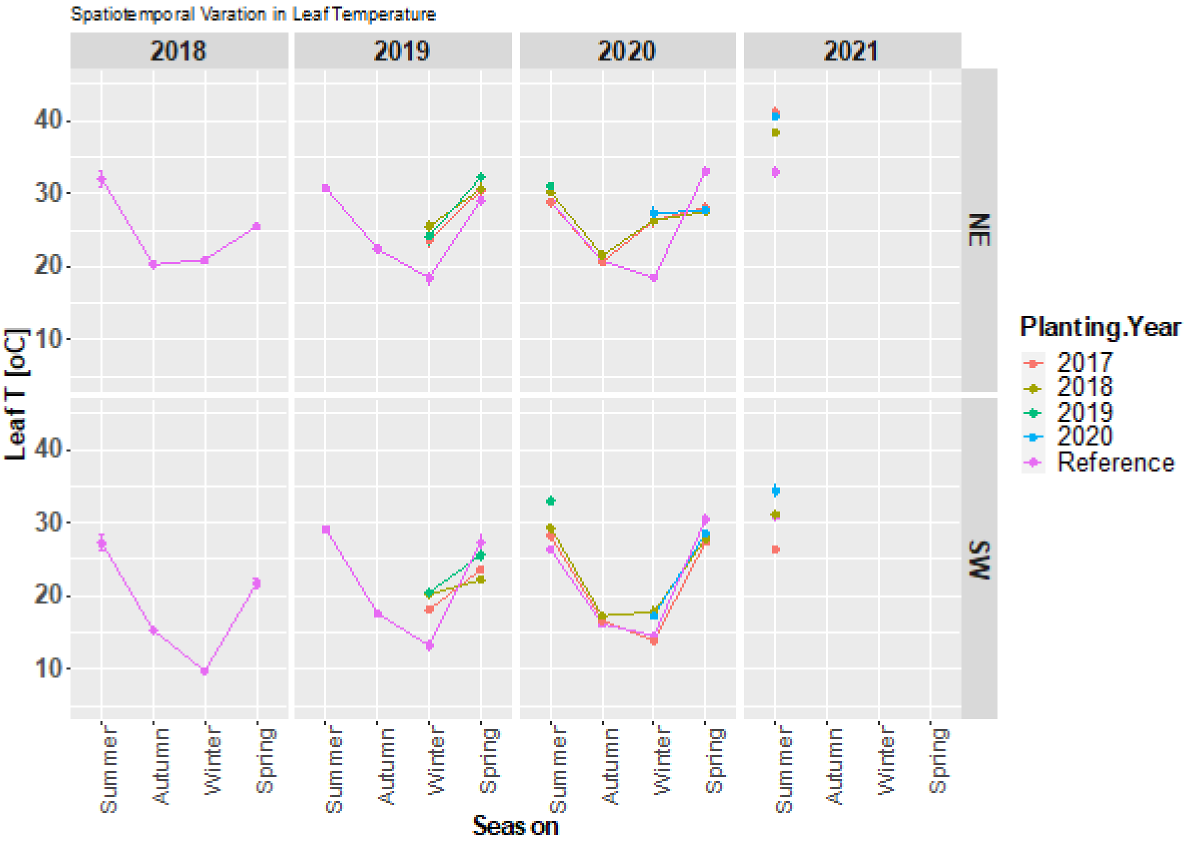


Figure 3.1.5b. Leaf temperatures (mean ± standard error) of *Tetratheca erubescens* in natural/ reference sites (NE-aspect plots:P7 and P25; SW-aspect plots: P3 and P5), and cuttings that were planted in translocation sites (NE-aspect: T23; SW-aspect: T6). The translocation sites contained cuttings that were planted in 2017, 2018, 2019 and 2020 Ecophysiological assessments in translocation sites commenced in the winter season of 2019 for cuttings that were planted in 2017-2019, and assessments commenced in the winter season of 2020 for the 2020 translocations.

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-aspect were demonstrated elevated leaf temperatures compared to natural plants (Figure 3.1.5b). The recent summer in 2021 demonstrated the highest leaf temperatures.

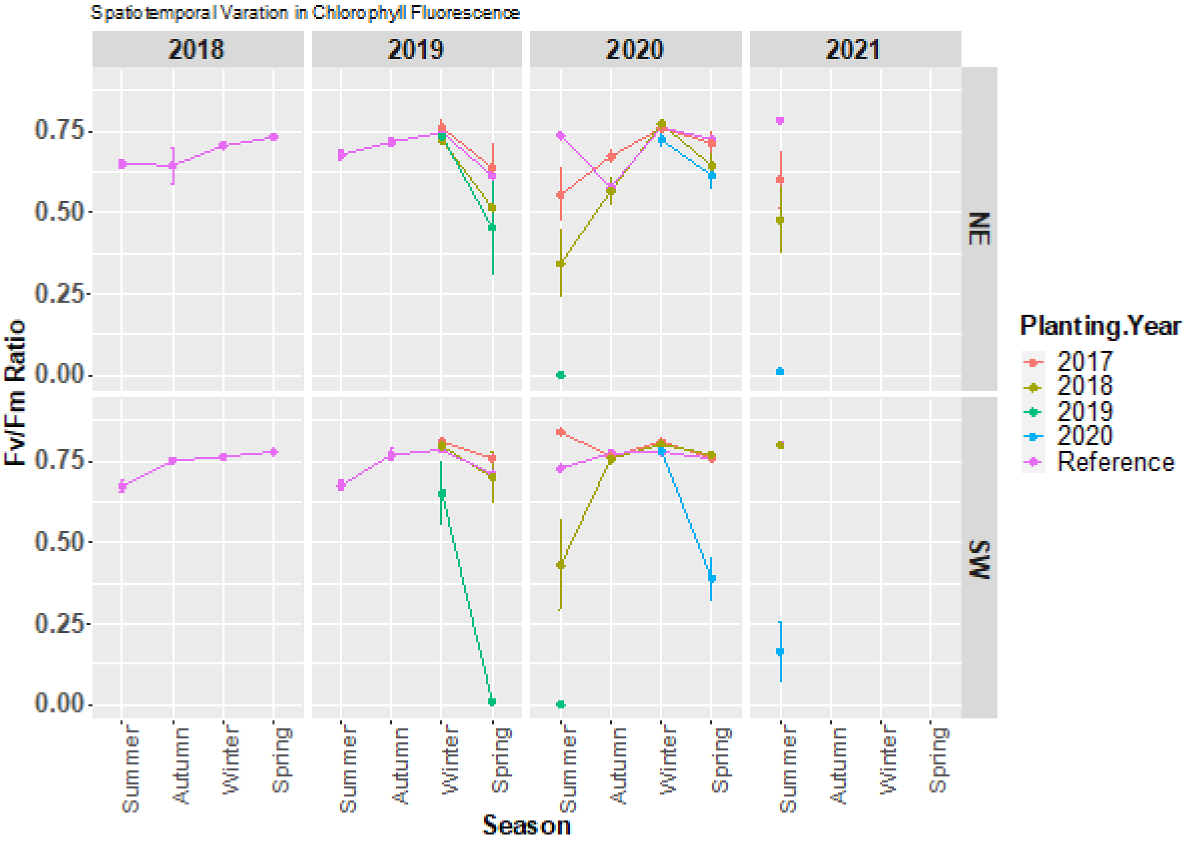


Figure 3.1.5c. Chlorophyll fluorescence (mean Fv/Fm ratio ± standard error) of *Tetratheca erubescens* in natural/ reference sites (NE-aspect plots:P7 and P25; SW-aspect plots: P3 and P5), and cuttings that were planted in translocation sites (NE-aspect: T23; SW-aspect: T6). The translocation sites contained cuttings that were planted in 2017, 2018, 2019 and 2020 Ecophysiological assessments in translocation sites commenced in the winter season of 2019 for cuttings that were planted in 2017, 2018 and 2019, and assessments commenced in the winter season of 2020 for the 2020 translocations.

Winter consistently represents the period of highest plant health across all sites, while spring and summer represent the periods of highest stress (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 functioning also coincided with increased mortality rates during this period.

Future research:

Ecophysiological assessments were also conducted on seedling derived greenstock that were planting in the 2019 and 2020 translocation – future analyses will determine the variation in performance of these propagules.

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:

- Assessment of soil chemical and physical composition analyses show 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, while BIF soils sampled underneath and adjacent to *T. erubescens* plants were associated with higher 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).

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

Research outcomes:

- Lower total N, P, K and Ca composition in leaves of *T. erubescens* and *B. arborea* compared to other species.
- Based on the sampling, there was separation of species sampled from the ridge top and slope locations, with BIF classified as overlapping due to sharing similar leaf tissue composition traits with both the ridge top and slope locations.
- 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).

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

Research outcomes:

- Soil treatments (as outlined in Elliott et al 2018, 2019), did not show improved establishment due to poor survival rates observed in Summer 2018 or Summer 2019.
- Two types of iron fertilizer were tested as soil treatments (2017 translocation: iron chelate supplement; 2018 translocation: Fetrilon Combi2).
- Details of research are in Annual Research Reports 1 and 2 respectively (Elliott *et al.* 2018; Elliott *et al.* 2019).

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

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

Soils were sampled in July 2017 and October 2017 from three locations and have been stored for assessment. Unforeseen circumstances have delayed the timeline for analysis, while an alternate service provider and their requirements for sample submission are confirmed. The continuation of this objective has been delayed and the revised timeline for analysis is Autumn 2021 (see Elliott *et al.* 2019 for details).

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

Soils were sampled in July 2017 and stored for assessment (as described in Elliott *et al.* 2019). The continuation of this objective has been delayed and the revised timeline for analysis is in Spring 2021.

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

Soils have only been collected from natural sites (as described in Elliott *et al.* 2019) and stored for assessment. The collection of soils from translocated sites will occur later in the project as translocations mature (see Table 4 of the Program Schedule).

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

Research outcomes:

- Initial soil inoculation trials in the 2017 translocation experiment did not support improved establishment and growth of planted cuttings in the different translocation sites.
- Details of research are in Annual Research Report 1 (Elliott *et al.* 2018).

PROGRAM SCHEDULE

Project management schedule

A five-year project is underway, with components varying in start date and period as described in Table 4. Translocation and monitoring, and plant function analysis are proposed for each year, with seed biology concentrated in the first years and soil nutrition and biological function studies both at the start and towards the end – reflecting their focus on initial conditions and on conditions in developing translocated populations.

Table 4 Program schedule.

	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						▨	▨													

Acknowledgements

Kings Park Science wishes to acknowledge the substantial input of Terry Netto, Anne-Louise Vague, Sam Tonkin and Corrie Watts in assisting with onsite logistics and research support over the year. Thanks to Susan Whiteley (Kings Park Science, DBCA), Emily Tudor (Curtin University), Eloise Ashton (The University of Notre Dame) Luisa Ducki (Murdoch University), Lauren Svejcar (Murdoch University) and Austin Guthrie (Curtin University) for assisting in translocation installations, seed collections, laboratory and field work. The Friends of Kings Park and the entire BGPA horticultural staff are also thanked for assisting at critical points with propagation work in between other commitments and responsibilities.

REFERENCES

Baskin CC, Baskin JM (2014) 'Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination.' 2nd edn. (Elsevier).

Bewley JD, Bradford KJ, Hilhost HW, Nonogaki H (2013): Seeds: Physiology of Development, Germination and Dormancy. 3rd Ed. New York, Springer.

Bradford KJ (2002): Applications of hydrothermal time to quantifying and modelling seed germination and dormancy. Weed Science, 50: 248–260.

Bureau of Meterology (2021) 'Climate statistics for Australian locations.' (Commonwealth of Australia) Available at http://www.bom.gov.au/ [verified 28 February 2021].

Elliott C, Turner S, Miller B (2017) *Tetratheca erubescens* Restoration Project (2016-17) Final Report. Report to Cliffs Pty Ltd by Kings Park Science, Botanic Gardens and Parks Authority.

Elliott C, Lewandrowski W, Turner S, Krauss S, Merritt D, Miller B, Stevens J (2018) *Tetratheca erubescens* Translocation Annual Research Report 1. Prepared by Kings Park Science, Biodiversity and Conservation Science in the Department of Biodiversity, Conservation and Attractions for Mineral Resources Ltd.

Elliott C, Lewandrowski W, Turner S, Krauss S, Merritt D, Miller B, Stevens J (2019) *Tetratheca erubescens* Translocation Annual Research Report 2. Prepared by Kings Park Science, Biodiversity and Conservation Science in the Department of Biodiversity, Conservation and Attractions for Mineral Resources Ltd.

Elliott C, Lewandrowski W, Turner S, Krauss S, Merritt D, Miller B, Stevens J (2020) *Tetratheca erubescens* Translocation Annual Research Report 3. Prepared by Kings Park Science, Biodiversity and Conservation Science in the Department of Biodiversity, Conservation and Attractions for Mineral Resources Ltd.

Hardegree SP, and Emmerich WE (1992) Effect of matric-priming duration and priming water potential on germination of four grasses. Journal of Experimental Botany 43:233-238.

Krauss SL, Anthony JM (2019) The potential impact of mining on population genetic variation in the Banded Ironstone Formation endemic Tetratheca erubescens (Elaeocarpaceae). *Australian Journal of Botany* **67**, 172-182.

Michel B (1983) Evaluation of the water potentials of solutions of polyethylene glycol 8000 both in the absence and presence of other solutes. Plant Physiology 72, 66-70.

Miller B (2015) *Tetratheca erubescens* habitat study. Report to Cliffs Asia Pacific Iron Ore Pty Ltd by Kings Park Science, Botanic Gardens and Parks Authority.

Stock E, Standish RJ, Muñoz-Rojas M, Bell RW, and Erickson TE (2020) Field-deployed extruded seed pellets show promise for perennial grass establishment in arid zone mine rehabilitation. Frontiers in Ecology and Evolution 8.

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.

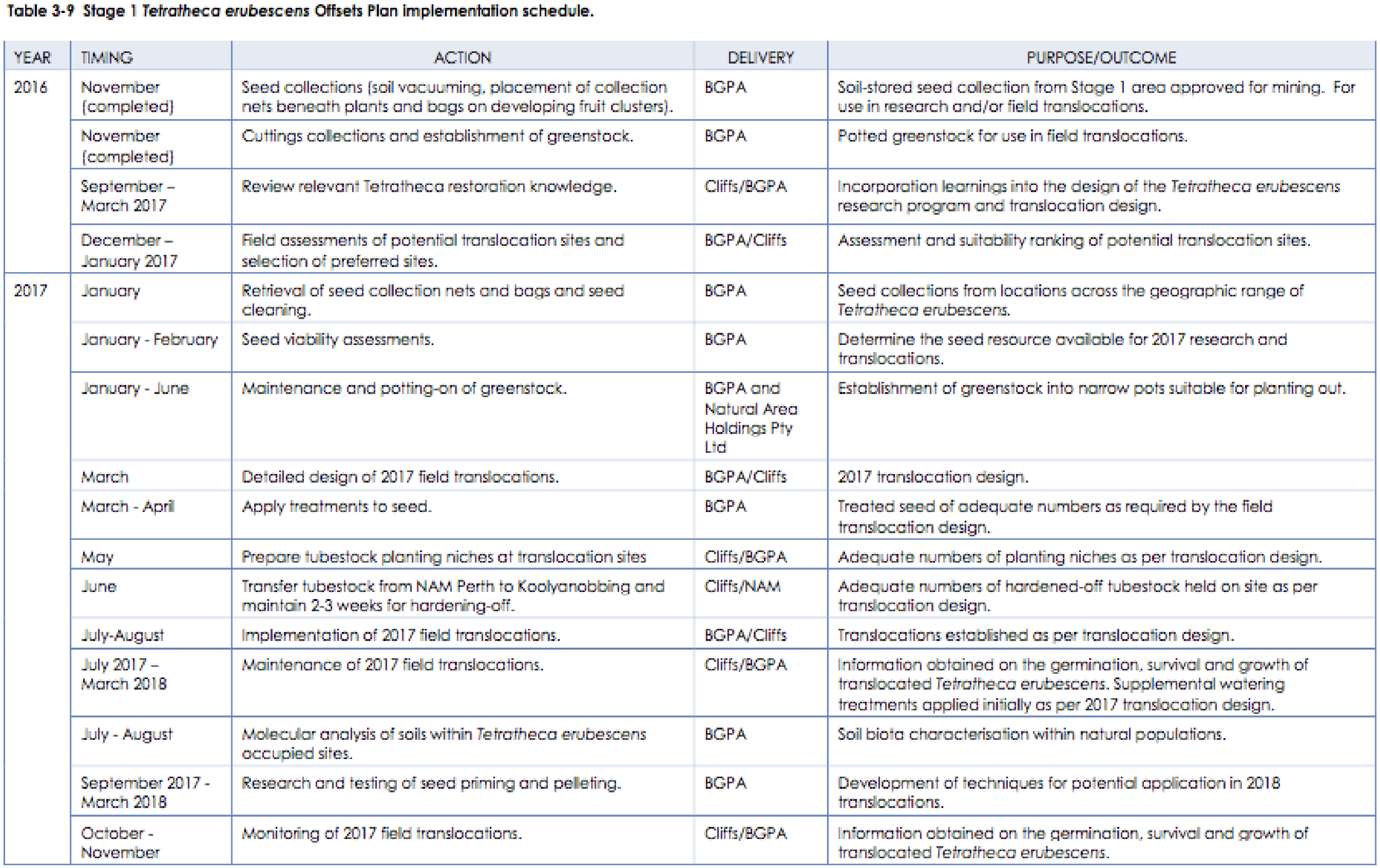
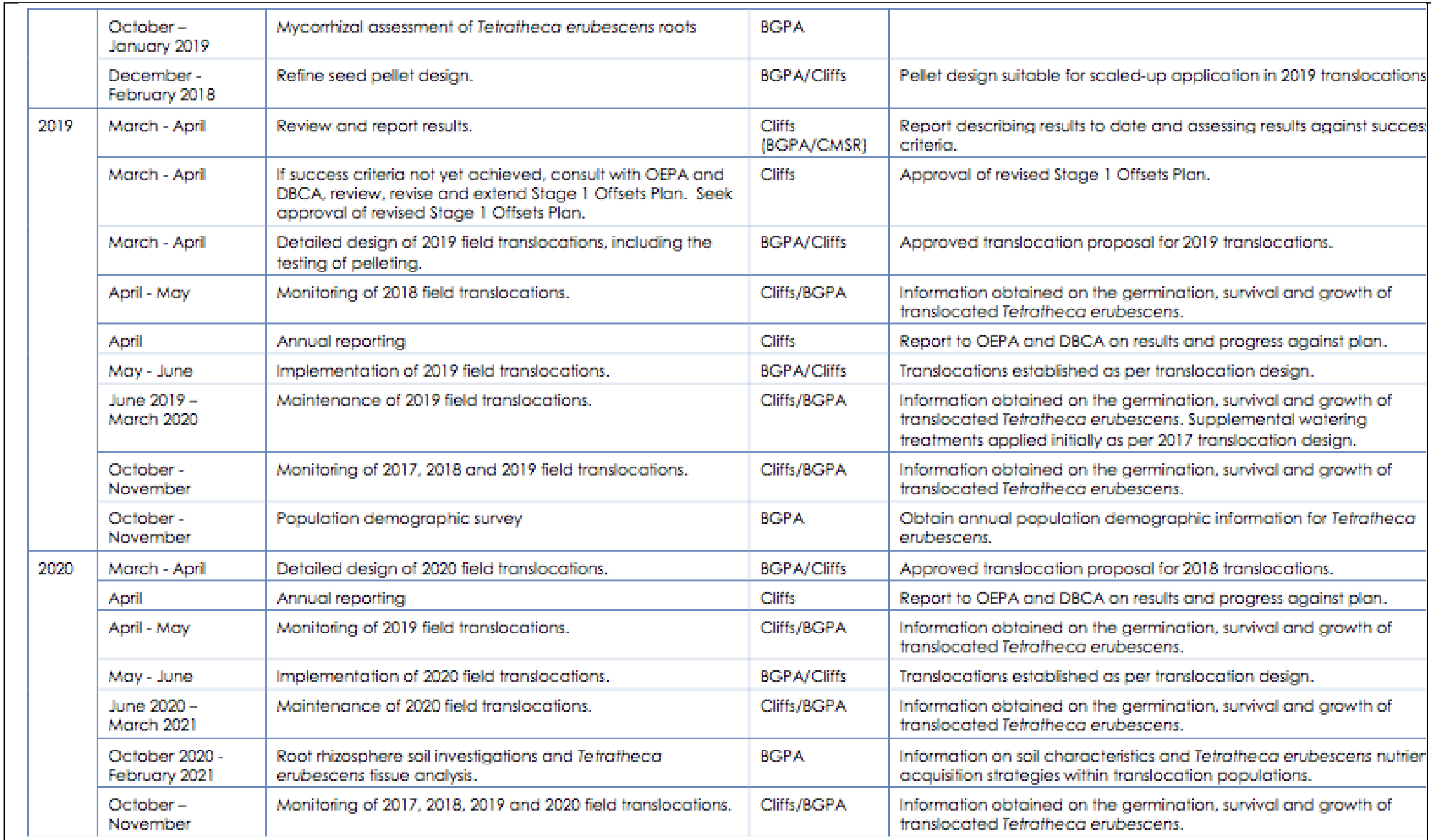


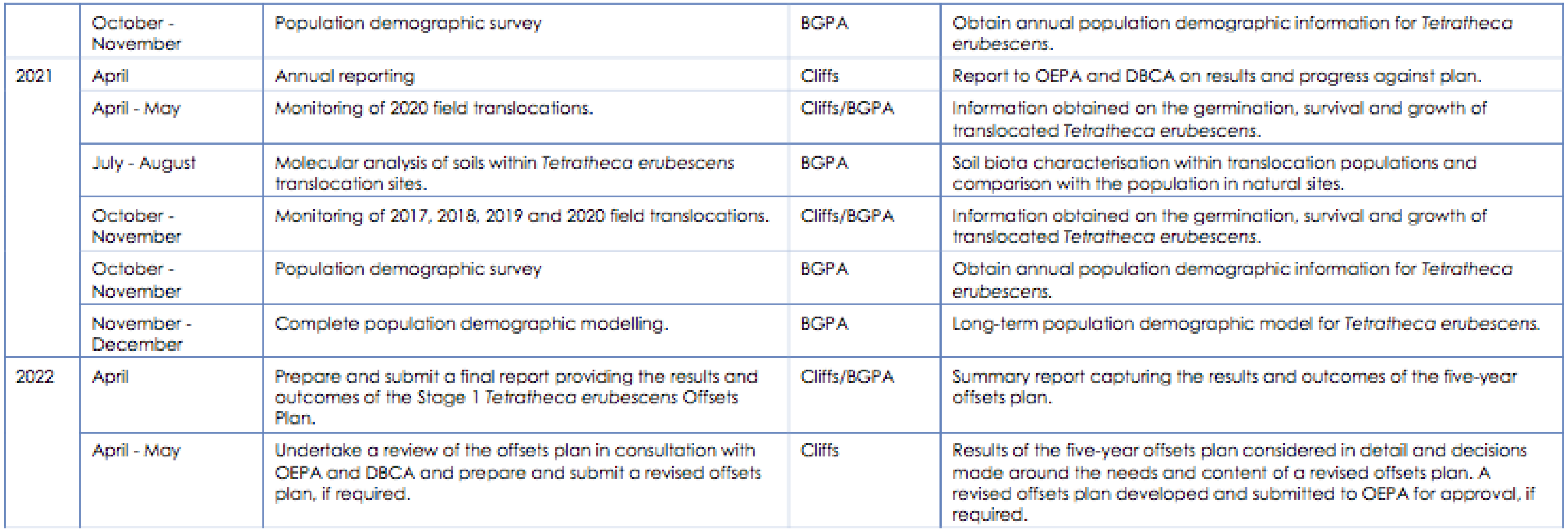
Table 3-9 Stage 1 *Tetratheca erubescens* Offsets Plan implementation schedule.

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.	In progress (Collected only)	Section 3 pp. 54
	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*	Completed (2018 report)	
	October 2017 - January 2018	Seed and cuttings collections.	BGPA	Obtain adequate viable seed and cuttings for 2018 research and translocations.	Completed (2018 report)	
	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.	Completed (2020 report)	
	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 individuals	In progress (2021 report)	Section 3 pp. 42-52
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.	Complete (2021 report)	Section 1 pp. 7-12
	February	Review and report results.	Cliffs/BGPA	Report describing results to date and assessing results against succ criteria.	Complete	
	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.	Complete	
	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.	Complete (2019 report)	
	March - April	Detailed design of 2018 field translocations.	BGPA/Cliffs	Approved translocation proposal for 2018 translocations.	Complete	
	April-May	Monitoring of 2017 field translocations.	Cliffs/BGPA	Information obtained on the germination, survival and growth of translocated *Tetratheca erubescens*.	Complete (2019 report)	
	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.	Complete (2019 report)	
	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.	Part complete (2019 report)	
	June - November	Preliminary field testing of pelleting methods.	BGPA	Determine if pelleting is likely to be suitable for field applications.	Complete	
	October - November	Monitoring of 2017 and 2018 field translocations.	Cliffs/BGPA	Information obtained on the germination, survival and growth of translocated *Tetratheca erubescens*.	Complete (2019 report)	
	October - November	Population demographic survey	BGPA	Obtain annual population demographic information for *Tetrathec* *erubescens*	Complete (2019 report)	
	October – January 2019	Seed temperature and water potential testing.	BGPA	Information to refine optimal seed pre-treatments.	Complete (2020 report)	
	October – January 2019	Seed and cuttings collections.	BGPA	Obtain seed and cuttings for 2019 translocations.	Complete (2020 report)	



	October – January 2019	Mycorrhizal assessment of *Tetratheca erubescens* roots	BGPA		Incomplete	Section 3 pp. 54
	December - February 2018	Refine seed pellet design.	BGPA/Cliffs	Pellet design suitable for scaled-up application in 2019 translocations	Complete (2021 report)	Section 1 pp. 11-12
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.	Part complete (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*.	Complete (2021 report)	Section 2 pp. 13-19
	May - June	Implementation of 2020 field translocations.	BGPA/Cliffs	Translocations established as per translocation design.	Complete (2021 report)	Section 2 pp. 13-19
	June 2020 – March 2021	Maintenance of 2020 field translocations.	Cliffs/BGPA	Information obtained on the germination, survival and growth of translocated *Tetratheca erubescens*.	Complete (2021 report)	Section 2 pp. 35-39
	October 2020 - February 2021	Root rhizosphere soil investigations and *Tetratheca erubescens* tissue analysis.	BGPA	Information on soil characteristics and *Tetratheca erubescens* nutrier acquisition strategies within translocation populations.	In progress	Section 3 pp. 53-54
	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 (2021 report)	Section 2 pp. 13-19

	Complete (2021 report)	Section 2 pp. 28-34

	October - November	Population demographic survey	BGPA	Obtain annual population demographic information for *Tetratheca erubescens*.
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*.
	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.
	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*.
	October - November	Population demographic survey	BGPA	Obtain annual population demographic information for *Tetratheca erubescens*.
	November - December	Complete population demographic modelling.	BGPA	Long-term population demographic model for *Tetratheca erubescens*.
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.
	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.

Date	**Activity**	**Details**
June/July 2020	Presentation: Project updates	Update on the status of the *Tetratheca erubescens* project provided to MRL and Strategen Environmental.
July 2020	Presentation: for Goldfields Threatened Species Recovery Team	Update on the monitoring of *Tetratheca erubescens* and *Ricinocarpos brevis* translocations provided to Goldfields Threatened Species Recovery Team meeting with MRL