Doyle, C.A.T., Pellow, B.J., Rapmund, R.A., Ooi, M.K.J. (2021) Preparing threatened plants for translocation: does home soil addition and nutrient loading improve growth and flowering? *Plant Ecology* Vol. 222, Pp 829–842. The final publication is available at Springer via https://doi.org/10.1007/s11258-021-01146-0

Preparing threatened plants for translocation: Does home soil addition and nutrient loading improve growth and flowering?

Chantelle A.T. Doyle[1,2*], Belinda J. Pellow[2], Ross A. Rapmund[3], Mark K.J. Ooi[1]

[1]Centre for Ecosystem Science, School of Biological, Earth and Environmental Sciences UNSW, Sydney, NSW 2052, Australia; [2]AMBS Ecology and Heritage Pty, Sydney, NSW 2050, Australia; [3]Hornsby Shire Council, Sydney, NSW 2077, Australia

***Corresponding author:** Centre for Ecosystem Science, School Biological Earth and Environmental Sciences UNSW Sydney NSW 2052 Australia T: +61 (2) 93852066 E: chantelle.doyle@student.unsw.edu.au

Keywords: augmentation, whole soil, threatened flora, propagation, provenance, nursery standards, reintroduction

OrcID: Chantelle Doyle 0000-0001-5102-3710, Mark Ooi 0000-0002-3046-0417

Published online 6th June 2021: Doyle, C.A.T., Pellow, B.J., Rapmund, R.A. and Ooi M.K.J. (2021) Preparing threatened plants for translocation: does home soil addition and nutrient loading improve growth and flowering?. *Plant Ecology* 222, 829–842.

Abstract

Translocation of threatened plants is increasingly being used as a conservation or mitigation action. The success of this practice is mixed and methods to increase likelihood of success are commonly investigated. Using a long-lived perennial shrub endemic to the Sydney Basin, Australia, as a case study, we examined the role of pre-planting nutrient loading (High, Low) and addition of Provenance (home soil) on growth and flowering, where Provenance soils had on average 50% lower nutrients than the Low treatment. We found that Provenance and Low treated plants grew better under propagation compared to High treatments, but these differences did not persist. At 11 months post planting, Provenance treated plants had growth rates no different from any other treatments and that plants under both High and Provenance soil treatments had higher peak flowering events, indicating that Provenance treated soils could confer a flowering advantage akin to fertilisation. This study demonstrates that there were no negative effects of growing plants using home soil, despite a lower nutrient status than standard propagation medium. Translocations, particularly reintroduction

27 or augmentation, should consider home soil treatment within pilot studies as a simple and cost-effective
28 method of potentially reducing transplant shock, providing ethical and phytosanitary measures are addressed.

29 **Acknowledgements**

30 This paper is dedicated to Lutz Jerofke and to Ingrid Liermann, keen naturalists and co-discoverers of one *H.*
31 *spanantha* population. We acknowledge the invaluable support of-Hornsby Shire Council and Community
32 Nursery staff and volunteers, particularly Mark Hood, Simon Dunne and Mary Warner. We also acknowledge
33 Erica Mahon, NSW Saving Our Species, the staff and Directors of AMBS Ecology and Heritage for their support
34 of this project. Thank you also to Associate Professor Tina Bell and Danica Parnell, University of Sydney, for
35 assistance with preliminary soil analysis and mycorrhizal staining and to Stephen Young of the NSW Soil
36 Conservation Service for soil collection.

37 **Declarations**

38 **Funding** Research has been conducted as part of the National Environmental Science Programme's Threatened
39 Species Recovery Hub (Project 1.3) and funding has been provided by the New South Wales Government
40 Saving Our Species program

41 **Conflicts of interest/competing interests** The authors have no conflicts of interest to declare that are relevant
42 to the content of this article

43 **Ethics approval** Planting and implementation were undertaken with a NSW Scientific Licence, approved by the
44 New South Wales Department of Planning, Industry and Environment.

45 **Availability of data and material** Data available on Figshare

46 **Code availability** Not applicable

47 **Authors contributions** Belinda Pellow and Chantelle Doyle (early career) developed the study concept and
48 design. Data collection and material preparation, including maintenance of plants, was performed by Chantelle
49 Doyle and Ross Rapmund. Data analysis was conducted by Chantelle Doyle and Mark Ooi. The first draft of the
50 manuscript was written by Chantelle Doyle. All authors commented on and contributed to previous versions of
51 the manuscript. All authors read and approved the final manuscript.

Introduction

Global pressures from urbanisation and a changing climate are increasing the need for a suite of *in situ* and *ex situ* conservation tools to protect biodiversity. Translocation of threatened plants is one such tool and is defined as the movement or direct transport of plants or plant material from one area to another for conservation or mitigation purposes, in order to benefit a species or ecosystem (IUCN 2013). Despite its increasing popularity (Silcock et al. 2019), this process is resource demanding and the success rates uncertain (Godefroid et al. 2011; Dalrymple et al. 2012; Guerrant Jr 2013). Translocation guidelines have therefore been developed to introduce practices that improve establishment success rate, and generally converge on several key factors including understanding of species ecology and biology, propagation, planting and long term maintenance and monitoring (Falk et al. 1996; Maschinski & Albrecht 2017; Commander 2018).

Pre-planting actions used to facilitate translocation successes are numerous and can include confirmation of best propagule type (Guerrant Jr & Kaye 2007), identification of planting sites with critical pollinators (Reiter et al. 2017) and host plants (Lawrence & Kaye 2008), site preparation (Godefroid et al. 2011), inoculation with generic laboratory culture and species symbiotic mycorrhiza (Zubek et al. 2009; Reiter et al. 2018), germination optimisation (Cochrane et al. 2002), selective propagation (Godefroid et al. 2016) and population mixing based on optimum population genetics (Shapcott et al. 2009; Cuneo et al. 2018; Van Rossum & Raspé 2018) or genetic home site advantage (Montalvo & Ellstrand 2000). However nursery propagation treatments, such as the application of fertiliser (nutrient loading), or the addition of small amounts of ‘home soil’ (whole soil) as an inoculation medium, have only been investigated in a handful of translocation studies (Fisher & Jayachandran 2002; Zimmer 2016; Brancaleoni et al. 2018; Michaelis & Diekmann 2018; Ruisi-Besares 2019). This is despite the fact that the effects of growing media are commonly investigated in commercial silviculture, horticulture and forest restoration studies (Salifu & Jacobs 2006; Salifu et al. 2009; Jacobs et al. 2015).

Nursery based nutrient loading has been shown to facilitate growth and nutrient storage in woody perennial seedlings (mostly silviculture and forest restoration studies of northern hemisphere *Populus, Quercus* and *Pinus* species), subsequently improving transplant competitive ability and stress resistance with the consequence of reduced post-planting care requirements (Timmer 1997; Casselman et al. 2006; Schott et al. 2016). Nitrogen concentration, particularly, has been linked with higher drought resistance and frost tolerance due to a suite of changes in functional attributes including higher root and shoot growth, greater stem diameter and greater above and below ground biomass (Fernández et al. 2007; Oliet et al. 2009; Oliet et al. 2013; Schott et al. 2016). These effects appear to be strongest when loading occurs pre-planting (usually in autumn) (Oliet, Puértolas et al. 2013, Schott, Snively et al. 2016), however results are heavily influenced by species, region and climate, particularly in xeric environments. Several studies have demonstrated inconsistent impacts of fertilization on growth and survival (Cuesta et al. 2010; Trubat et al. 2010), or results in which nutrient deprivation (nutrient hardening) in the late stages of growth favours survival, because of reduced above ground biomass, reduced leaf size, and higher water use efficiency (Trubat et al. 2011). Inconsistencies in post-planting response to nutrient loading have been mirrored in horticultural plants, particularly where transplanted to semi-arid environments (Franco et al. 2006).

In naturally nutrient-poor regions, such as many parts of Australia, nursery based nutrient loading research for restoration and plant transplant benefit is uncommon. Field application of fertiliser for restoration and silvicultural purposes does occur, often resulting in varied responses driven by environmental conditions (Stoneman et al. 1995; Rokich & Dixon 2007), but clear guidelines for, or an understanding of the positive or negative impacts of nutrient application pre-planting are limited. Many Australian plant species are known to be negatively impacted by artificially increased nutrient levels *in situ*, most often from residual fertiliser and run-off (Thomson & Leishman 2004 and the references therein), with some particularly P sensitive species suffering reduced biomass accumulation as a result (Standish et al. 2007).

Promoting mutualistic relationships with soil microbiota, such as mycorrhiza and rhizobacteria, at the pre-planting stage has been found to increase establishment success and reduce transplant shock in threatened plant translocation (Haskins & Pence 2012). Mutualistic relationships afford plants access to limited nutrients, particularly phosphorous and nitrogen, and micro nutrients such as zinc and copper (Barea & Jeffries 1995), but also confer greater absorption of water to afford drought and pathogen resistance (Haskins & Pence 2012 and the references therein). Within low phosphorous systems, such as Australia, these relationships are critical for increasing root area and ability for P uptake (Handreck 1997) and have increasingly been used within commercial horticulture to increase plant establishment and flowering post planting (Baum et al. 2015). The soil biota is also influential in determining plant community structure and assemblage (Wardle et al. 2004). For management of threatened flora, inoculation is sometimes critical for propagation of species with symbiotic associations such as orchids (Batty et al. 2001; Reiter et al. 2018), and many other species have mycorrhiza specific relationships based on geographic, climatic and edaphic constraints (Gemma et al. 2002; Bothe et al. 2010; Reiter et al. 2013).

Home soil inoculation is one method of introducing species or location specific microbiota and recent studies have shown that it can provide similar results to the application of generic rhizobacterium cultures for some species (Michaelis & Diekmann 2018). Home soil may contribute potentially unidentified co-associations, such as helper bacteria like *Pseudomonas* (Duponnois & Plenchette 2003), increase plant tolerance to biotic stress and immune response to pathogens (Chialva et al. 2018), expedite acclimatisation to the home environment before planting, and can reduce the risk of microbial competition post-planting as may occur with commercial plant growth promoters (Haskins & Pence 2012). Conversely, home soil inoculation can also potentially introduce soil borne pathogens (Mendes et al. 2013 and the references therein). The use of home soil inoculation as part of pre-planting translocation planning is uncommon.

Because of the resource demands and the need to secure adequate funds for implementing translocation of threatened species (ACT 2017; Commander 2018), methods to increase the likelihood of translocated plant survival and reproduction, including production and planting, must be tested. Using the Critically Endangered species *Hibbertia spanantha* as a case study, a long-lived perennial subshrub endemic to the Sydney Basin Bioregion in temperate Australia, we sought to examine the impact of pre-planting nutrient loading and home soil application on plant growth and flowering (as an early indicator of reproduction) post-translocation. Use of fertilisation for propagation of threatened plants has generally focused on growth response under provision of traditionally limiting nutrients, often phosphorus (Fisher & Jayachandran 2002; Gemma et al. 2002), but these impacts are rarely examined under translocation scenarios. Specifically, we asked:
(i) How does increased nutrient loading, compared to home soil addition, affect seedling growth under nursery conditions?
(ii) Does pre-planting treatment affect growth and flowering of individuals post-planting?

Methods

Study region and species

Hibbertia spanantha Toelken & A.F. Robinson is a decumbent to sprawling subshrub to approximately 30 cm high (Supplementary material 1). It is a member of the Dilleniaceae, a family of mainly shrubs, with 11 genera, of which the genus *Hibbertia* is known to have vascular-arbuscular mycorrhizal associations (VAM) associations (Brundrett & Abbott 1991). Described as growing in dry open forests, *H. spanantha* is generally associated with shale sandstone transition soils (DPIE 2020) within the Sydney Basin Bioregion. The species was originally known from only two populations in 2013 when cuttings were first collected for propagation, expanding to a total of four populations by 2019. The largest population contains 89 stems, some of which are suspected ramets. The smallest population is restricted to one plant. Anecdotal evidence suggests that *H. spanantha* is clonal, and the small populations may also consist of ramets. Each population is highly fragmented, with an area

of occupancy (AOO) no greater than 100m^2. Its restricted range, small population size, low area of occupancy and the low observed recruitment of most populations, compounded by threats from urbanisation in the Sydney Basin, have resulted in a listing of Critically Endangered (Commonwealth EPBC Act 1999 and NSW BC Act 2016) and within the 100 Australian species at most risk from extinction (Silcock 2018). Due to this status, *H. spanantha* is the subject of a translocation project with an aim to augment the current distribution.

The study site for planting individuals was a small urban reserve fragment in the northern suburbs of Sydney, New South Wales, Australia, with translocation sites located no greater than 20m from the known wild population, comprising eight extant plants. Vegetation is described as Sydney Coastal Dry Sclerophyll Forest (Keith 2006) and the site receives an average annual rainfall of 1079mm (BOM 2019), distributed relatively evenly across the year, with higher falls in summer and autumn. Potential planting locations were chosen based on proximity to wild plants and perceived similar microclimate; the parent plants being observed to persist in canopy gaps.

Propagation and nutrient application

The production of seeds in *H. spanantha* is sporadic and, within some populations, viability is thought to be low based on preliminary testing by the Australian PlantBank. In 2013 the Australian Botanic Garden Mt Annan established an *ex situ* collection of *H. spanantha* based on cuttings of three plants from the study population, which at that time was believed to contain only five plants. The total population was revised to eight by 2016, following additional searches. Propagation material for our experiment was sourced from this *ex situ* collection and, like the extant wild population, is likely to be from a single genetic lineage. Propagation occurred in a local NIASA (Nursery Industry Accreditation Scheme Australia) and EcoHort certified nursery (Australian Environmental Management certification). Cuttings of 100mm length were taken on 7 June 2017 from semi-hard wood material with 50% of the leaf material removed from the base of individual cuttings and struck using a two-stage hormone treatment (Esi-root [Esiroot, Chatswood, NSW

Australia] 20 minute immersion soak and Clonex Purple [Yates, Clayton, VIC Australia]) applied to the base 15mm. Cuttings were struck in a vermiculite/perlite propagation mix in punnets and were stored in greenhouses with controlled base heat applied at 22°C and regular misting periods during daylight hours. Striking was first observed on 16 August 2017 and plants were transferred to potting medium on the 31 August 2017.

A customised potting medium of organic (composted pine bark and coir peat) and inorganic matter (fine sand) was prepared, identified by the nursery to maximise growth in Australian native species and based on ratios outlined in Handreck et al. (2002). This growth medium conforms with Australian Standards (AS 3743, 2007) of physical structure to balance porosity and drainage, water holding capacity and promote root growth. Into 160L of standard growth medium, 384g of the commonly used control release fertilizer (Green Jacket No.5 [Australian Growing Solutions, Tyabb, VIC Australia]) was added, which contains low P suitable for Australian native species. Ratios of nutrients within Green Jacket are described as NPKS 20:1.5:9:1.5. An additional 30g of Yate FlowTrace (Yates, Clayton, VIC Australia) was combined with the 160L of potting media to provide trace elements, particularly Fe.

Our experimental design involved growing plants in three types of media; a high nutrient mix (High), a low nutrient mix (Low) and a treatment that included addition of Provenance (home) soil mix.

To prepare different levels of nutrient loading (High and Low), additional control release fertilizer (Green Jacket) was added to the standard potting medium (approx. 7.5g for High and 3.5 g for Low). For the Provenance soil treatment mix, soil was collected from the O and A horizon (top 15 cm) within the root zone of the wild plants, one week prior to potting. Soil was sieved to 5mm, to remove large roots and leaf litter. Home soil was then mixed at 50:50 ratio with standard potting medium. Mean nutrient levels of High, Low and Provenance soil treatments used for the experiments are shown in Table 1.

A total of 25 plants were available for the experiment and were transferred as struck cuttings directly into pre-mixed potting medium. We acknowledge that threatened species research is often hampered by smaller sample sizes and that results therefore need to be interpreted with this in mind. Ten plants were potted in 125ml forestry tubes filled with the High nutrient level treatment and a further 10 with the Low treatment. The remaining five plants were potted into tubes containing the Provenance soil mix. Fertiliser and provenance soil mixes were reapplied across all treatments at the same ratios in February 2018, during repotting.

Soil analysis

To assess variation in soil characteristics field soils from the parent population (within 5m of wild plants) were collected with a hand auger to depth of the soil parent material (i.e., sandstone). Field characteristics (texture, colour) and pH were used to determine the soil description. Field soils from the parent site and potting medium (nursery mixes including home soil mix) were oven dried. Chemical analysis was conducted on both potting medium (n=24) (collected in February, five months after plants were potted) and field soils (n=24) (top 15cm only) by commercial laboratory CSBP and included nitrogen (Ammonium and Nitrate mg/kg), Phosphorous Colwell (mg/kg), Potassium Colwell (mg/kg) and Organic Carbon (%).

Field planting design

Propagated plants were installed in a randomised block design in April 2018, with five individuals planted randomly in each of five 1m x 1m blocks at a field site spanning a linear range of approximately 100m; blocks were named Upper, Lower, Compound, Central and Burnt. Each block was within 20 metres of a wild population and included two plants from each of the High and Low nutrient soil treatment and one from the Provenance soil treatment. In one block, this was reduced to four plants due to senescence in the nursery of a plant under the High treatment. Plants were labelled with aluminium tags. Note that each block was similar, although the one labelled Burnt had

been subject to fire seven years prior to the experiment (and was colloquially known as the burnt area).

Plants were watered in by hand using 5L per plant. Follow up watering was undertaken at a rate of 4L per plant every two days for the first week, then 4L every three days for the second week. Plants were then hand watered weekly for the next month, at a rate of 3L per plant. After initial establishment, watering was undertaken by hand on an as needs basis (when soil was no longer damp to the touch and there was observable plant stress of drying leaves and tip wilting). Due to an unseasonably dry winter, frequent hand watering was required; over the course of 12 months plants received a minimum of 115.5L per plant by hand and an additional 1220ml from natural rainfall (rain gauge 1.8km from the site). Due to this extreme heat and water stress, all translocated plants exhibited leaf desiccation and leaf drop during summer and some senesced.

Mycorrhizal infection

Fine roots were collected during repotting in February 2018 prior to planting (5 months after original potting) from all 24 plants. Roots were cleared and stained based on methods from Brundrett et al (1996). When necessary, roots were cleared using KOH (10%) in a water bath for approximately 2 h. Fungal hyphae in roots were stained with Trypan Blue (1%) dissolved in lactic acid: distilled water: glycerol in the proportions 2:1:1 for 24 h. A solution of lactic acid: distilled water: glycerol (2:1:1) was used for de-staining and storage. Sub-samples of roots were viewed under magnification using a compound microscope to determine the presence of internal hyphae and vesicles. Infection was scored (0-3), 0 being absent and 3 being near total fungal colonisation of root.

Plant growth and flowering

Pre-planting growth was assessed in the nursery environment fortnightly for 7 months (223 days). Growth parameters measured were height (h) of tallest branch (mm) and width (mm) measured tip to tip. Secondary measures of orthogonal widths (mm) were also collected, but as the species grows most consistently along one axis, orthogonal width was subsequently not considered a reliable

representative growth metric. Plant growth post-planting was monitored monthly for 11.5 months (358 days). At the end of nursery propagation, it became evident that height was no longer a consistent metric due to the sprawling growth pattern of this species as it aged, which is also common in other *Hibbertia* species (Cuneo et al. 2018). Measurements of growth in the field were therefore taken as width only, while height and width measurements were retained as the key metrics during pre-planting propagation.

Growth was calculated as the average height and width in the nursery and average width in the field. Comparisons of growth were made after 15 and 31 weeks for the nursery experiments, and 15 and 47 weeks for field growth experiments. Single measures of growth such as height or width have been found to correlate well with other metrics such as plant volume (Maguire & Menges, 2011). Relative growth rates (RGR) for each treatment were calculated at the same time points as growth comparisons, using "$(ln(g2) - ln(g1))/(t2 - t1)$" (Price & Munns 1999; Hoffmann & Poorter 2002), where g2 is the final width and g1 is the width at first measurement, t2 is time at final measurement and t1 is time of measurement commencement (in months). Height and width were used as non-destructive surrogates for biomass to calculate RGR, as is common practice in many studies of seedling and juvenile plant growth *in situ* (e.g.Menges et al. 2016).

Measurements of flowering were tracked post-field planting only and used as an estimate for reproductive potential. Initially measures were taken monthly and increased to between weekly and fortnightly during peak flowering (Nov-Jan). Flower production measurements were time of first flowering (date at which first open flower was recorded), peak flower number (highest flower production per plant) and time to 50% flower production (T50). T50 was calculated using a general dose response model (often applied to germination time) in the *drc* package in R (Ritz, 2015). Water stress and heat during summer affected most planted individuals, resulting in leaf drop and reduced flowering. Severely affected plants were removed from the analysis, likewise any dead plants.

Measurements of flowering and growth at 47 weeks post planting were therefore conducted on the remaining 17 plants. All prior measurements were conducted on 24 plants.

Chlorotic growth observed during plant propagation was scored ordinally according to severity of leaf yellowing; where plants with dark green foliage were perceived as healthy and scored at 0 and plants with severely yellow/white chlorotic foliage, indicative of stress were scored 3. Scoring continued until tube stock were planted into the field (i.e., 31weeks).

Statistical analysis

All statistical analyses and graphics were conducted using the statistical platform R (R Developmental CoreTeam 2019). Soil nutrient levels and growth (RGR and [log] width and [log] height at 15 and 31wks) of nursery plants were analysed using linear models (R base package), with soil treatment (with the three levels High, Low or Provenance mixes) as the fixed factor. Additionally, analyses of RGR were size-standardised by adding the starting dimensions of each plant as a covariate, as larger plants are known to have a lower RGR relative to smaller plants when measured over the same time period (Paul-Victor et al. 2010). Post-hoc tests of significance were conducted using treatment contrasts in the summary function in R (Crawley, 2007). Assumptions of normality were assessed via fitted vs residuals plot using base R *plot*.

For the response variables field growth ([log] width only at 15 and 47 weeks), peak flowering, time to first flower and T50, we used linear mixed models with a Gaussian distribution and default variance-covariance structure (unstructured) in the *lme4* package (Bates et al. 2015), again with soil treatment as the fixed factor, and block as the random factor to constrain any random influence between blocks. Note that due to near complete senescence in one block post-planting, analysis was reduced to plants in four blocks in the 47 week analysis. Linear mixed models were also used to test RGR (width) but with size at time of planting included as a covariate (as above). Due to decline of many individuals towards the end of the experiment from heat stress and drought, RGR for field

298 growth was only assessed at 15 weeks. Assumptions of normality were again assessed via fitted vs
299 residual plots and P values were calculated using the *lmerTest* package (Kuznetsova et al. 2017).
300 Posthoc tests of significance for the mixed models were made with Tukey pairwise comparisons
301 using glht in the *multcomp* package (Hothorn et al. 2008).
302
303 Degree of mycorrhizal infection and chlorotic foliage were scored ordinally and analysed with
304 Kruskal Wallis and Dunn's post hoc analysis using the *dplyr* package (Hadley Wickham et al. 2020).

305 Results

306 Soil nutrients

307 Nutrients levels varied considerably between replicates however there was a clear difference
308 between High and other treatments. High variation could be explained by differences in root
309 biomass between plants and initial investigation found more complex root structure under
310 provenance soil and variation in root development between nutrient treatments (unpubl. data). The
311 concentrations of Nitrogen (NH4 and NO3), P and K were significantly higher in the High treatment
312 mix compared to the home Provenance soil mix (Table 1) used for growing plants in stock tubes
313 (P=0.005 [NH4], P=0.02 [NO3], P=0.009 [K] and P=0.02 [P]). NH4 concentrations and K
314 concentrations were also significantly higher in High treatments compared to Low (P=0.002 and P=
315 0.02 respectively). Organic Carbon (C) was significantly higher in High treatments compared to Low
316 (P=0.04), but not Provenance treatments (Table 1). No significant differences were detected
317 between Provenance and Low treatments (Table 1).

318 For soils at each of the home planting sites, nutrient content was broadly similar. There was no
319 significant variation in concentrations of NO3, while Upper and Central had higher P values than
320 Compound. Upper also had significantly lower NH4 and significantly higher K compared to most
321 other locations. Noticeably, soils from the Burnt site only differed significantly from other sites in a
322 higher C content (Table S2).

Overall, nutrient levels were between 2.8x (K) and 250x (NO3) lower in the field than the nursery standard growth medium. Even Provenance soil treatment mixes from the nursery maintained nutrient concentrations at least double (or much greater) that of the home soil values (Table 1).

Growth during propagation

Mean growth (width) of plants after 15 weeks propagation differed significantly between soil treatments ($F_{(2,21)}$ =5.16, P=0.015). Post-hoc comparisons showed that plants grown under High nutrient treatments were significantly smaller than both Low (t=-2.92, P=0.008) and Provenance (t=-2.47, P=0.022) treatments (Fig 1a.). The RGR (width) mirrored these results and was significantly impacted by treatment at 15 weeks ($F_{(2,20)}$ = 6.8, P=0.006) where the rate of growth in both Low and Provenance treated plants was significantly higher than High treated plants (t=-3.33, P=0.004 and t=-2.92, P=0.009 respectively) (Fig 1b.). Height was also significantly different between treatments ($F_{(2,21)}$ = 4.83, P=0.019), with plants grown under High nutrient treatments being significantly shorter than those grown under Low (t=-2.8, P=0.012) and Provenance treatments (t=2.43, P=0.024). Treatment likewise significantly impacted RGR (height) ($F_{(2,20)}$ =7.8, P=0.002) which was lower in High relative to Low (t=-2.80, P=0.012) and Provenance treated plants (t=2.17, P=0.042). By 31 weeks there was no significant difference in growth between any treatment dimensions ($F_{(2,21)}$ =1.78, P=0.19 [width], $F_{(2,20)}$ = 0.98, P=0.39 [height]) or RGR (width) ($F_{(2,20)}$ =1.81, P=0.19). There was still a significant effect on RGR (height) ($F_{(2,20)}$ =7.05, P=0.005), driven by size at week one (t=-2.58, P=0.02), however RGR of High treated plants was not significantly greater than either Provenance (t=1.67, P=0.11) or Low (t=1.67, P=0.11).

Nutrient treatment also had a significant effect on plant health (χ^2 =10.6, P=0.005), which was measured by the average amount of chlorotic foliage. Plants grown under either High or Low nutrient treatments had significantly higher average scoring (± SE) of chlorotic growth compared to Provenance soil treated plants (2.11 ± 0.31, P=0.003 and 1.5 ± 0.31, P=0.034 respectively). Chlorotic

growth did not reoccur after the second nutrient application at 5 months, nor was it observed at all in the Provenance treated plants (Supplementary material 2).

Growth in the field post-planting

By 15 weeks post planting the growth (width) differences observed during propagation were not detectable between any treatment ($F_{(2,21)} = 0.92$, P=0.42). At 47 weeks post planting, Provenance and High nutrient treated plants tended to be wider than Low nutrient treated plants (Fig2b), however this pattern was not significantly different ($F_{(2,11)} = 3.36$, P=0.072). There were also no significant differences found for RGR at 15 weeks ($F_{(2,20)} = 0.94$, P=0.41) (data not shown).

Flowering post-planting

Treatment significantly impacted peak flowering ($F_{(2,10)} = 6.87$, P=0.013) where peak production was significantly greater in High treatments relative to Low (z=-3.49, P=0.001) and Provenance relative to Low (z=2.71, P=0.018) (Fig 3). There was no significant effect of treatment on T50 ($F_{(2,13)} =0.38$, P=0.69) or time to first flowering ($F_{(2,10)} = 1.09$, P=0.37).

Mycorrhizal infection

Roots of all treatments showed evidence of mycorrhizal infection, identified most often as branched hyphae (although it was not clear if it was internal or external). One plant of the 24 appeared to have no evidence of infection (a plant in the Provenance soil treatment). Infection in a further two plants (both High treatment) was indistinct, however the remaining high nutrient plants had definite mycorrhizal presence, possibly vesicles. Average scores assigned to the degree of infection (±SE) were 1.44 (±0.23) (High), 1.6 ± 0.22 (Low), 1.8 ±0.49 (Provenance). Anecdotally we also observed a greater root biomass and greater root maturation (identified by brown colour and hardening) in plants under Provenance compared to High treatments.

Discussion

Experimental assessment of nursery cultural practices designed to facilitate translocated plant establishment and buffer against initial transplanting shock are uncommon in the translocation literature. Despite general encouragement to consider planting environment by hardening prior to planting (Jacobs & Landis 2009), there is little correlation between nursery growing medium and intended recipient location, and research into home/whole soil propagation has only occurred in a handful of studies which generally focus on the introduction of beneficial soil microbes (Haskins & Pence 2012; Michaelis & Diekmann 2018; Ruisi-Besares 2019). This gap is particularly pertinent in systems where plants are subject to both nutrient and climatic stressors, such as Australia.

In our study of the threatened species *H. spanantha*, we found that High rates of fertiliser addition significantly reduced the growth and relative growth rates of plants during propagation, relative to Low and Provenance treatments. We also found that plant stress, assessed by the proportion of plants with chlorotic growth, was evident in all nutrient supplemented treatments, but not in plants grown using home Provenance soil mix. This outcome may be attributable to stress buffering attributes of home soil inoculated plants (Chialva et al. 2018), but conclusions are limited by the study design and further investigations into other potential drivers, such as texture and micronutrients, are required. The fact that Provenance treated plants had an equally high or highest RGR throughout propagation, along with soils treated at a reduced nutrient status, indicates that high nutrient status and optimal potting medium structure does not necessarily drive growth rates, at least for this species. While our results need to be interpreted with caution, due to the limited sample size, the evidence that nursery growing conditions can impact plant flowering in the first season post planting, with greater flower production in both High and Provenance treatments, highlights that addition of parent soil during early propagation may impart an establishment and flowering advantage distinct from nutrients alone.

Our findings, that nutrient loading did not significantly increase plant growth in the nursery, are in contrast to findings from other regions where native soils are more fertile (Oliet et al. 2009; Salifu et al. 2009). Broadly, nutrient loading in such systems is applied based on increasing plant resilience through nutrient (particularly N) facilitated biomass accumulation (Salifu & Jacobs 2006; Salifu et al. 2009; Grossnickle 2012; Jacobs et al. 2015). However, for Australian species and those of many other biodiverse hotspots around the world, soils are extremely nutrient poor (e.g. Hopper et al. 2015), and whilst there is general sentiment that many Australian native plants will respond positively to fertiliser application (to a point) (Leake 1993; Handreck et al. 2002), propagating plants for translocation back to nutrient-poor conditions presents a very different need. The fact that plants under Provenance treatments had a lower nutrient status than other treatments (but still at least double that of the home soil), and performed similarly to High nutrient treatments suggests both that nutrient addition has limited use in low nutrient ecosystems and that a home soil advantage may produce other benefits not tested in our study (Michaelis & Diekmann 2018).

The observed slower rate of tube-stock growth and chlorotic effects on all plants with nutrient addition, may be attributed to P toxicity, and was notably absent after the second application of fertiliser when plants were 5 months old. A potential mechanism explaining this was outlined by Tomson and Leishman (2004), who noted that even nutrient sensitive Australian plants were able to buffer or store excess applied nutrients if they were 6 or more months old. However, the ability to recover may depend on the species and on how close to the P toxicity threshold plants have reached (Groves & Keraitis 1976; Specht et al. 1977; Grose 1989).

One of our expectations regarding home soil application to nursery soil mixes was that it would increase mycorrhizal infection. The ability of mycorrhiza to buffer plants against biotic and abiotic stressors is well established in conservation biology and translocation (Barea & Jeffries 1995; Gemma et al. 2002; Bothe et al. 2010; Haskins & Pence 2012 and the references therein) and has been variously applied in commercial horticulture (Baum et al. 2015), particularly for transplanting

418 nursery cultivated plants to stressful environments (Franco et al. 2006). Mycorrhiza were detected in
419 all samples in our study, and we note the limitation that only coarse estimates of root infection were
420 made, which do not discriminate between beneficial or pathogenic associations. Observations of
421 different root structures, maturity and biomass between High and Provenance treatments indicate
422 growing medium impacted root development, but this was not assessed in our study. We suggest
423 further investigation to identify whether there is a mycorrhizal association in provenance sourced
424 soils and if this could infer a home site advantave, particularly for reintroduction or augmentation
425 translocations.

426 Achieving a successful translocation relies on two core factors; post planting establishment and
427 reproduction (Menges 2008; Monks et al. 2012; Godefroid et al. 2016). Our study used flowering as
428 an early analogue of reproduction and found no influence of treatment on T50 or time to first
429 flower, however peak flowering was impacted, where plants under High and Provenance treatments
430 displayed a higher peak than Low treated plants. This may be due to less resilience maintained by
431 plants originally treated at Low nutrient levels, which displayed a decline in size in the field towards
432 the end of the experiment, under significant drought effects. Corresponding lower flowering levels
433 may therefore be related to this loss of condition. Together, this indicates that Provenance treated
434 soils could also confer a reproductive advantage akin to fertilisation, because both High and
435 Provenance treated plants maintained growth and produced similar flowering levels. In projects with
436 limited timelines or budgets, expediting flower production (and potentially recruitment and
437 establishment of a self-sustaining population) could significantly improve likelihood of long-term
438 translocation success.

439 Based on the resource demands of translocation as a conservation tool, factors which may increase
440 the likelihood of plant survival and reproduction should be incorporated at all stages, including
441 propagation. Although limited by small sample size due to the inherent problems of using
442 threatened species, our study demonstrates that plants from nutrient poor regions can be

propagated under lower nutrients than conventionally employed, where they are co-propagated with home soil, and that nutrient loading influences peak flowering and may impact relative growth rates at least in the first season post planting. Although the influence of mycorrhiza and other co-beneficial associations in home soil is, at this stage unclear, it caused no detriment, and we recommend that where phytosanitary concerns can be controlled (and a thorough risk assessment be undertaken including soil testing for diseases such as phytophthora if required) home soil be incorporated as part of a well-designed translocation pilot study. We suggest further investigation of the potential for use of this technique is warranted, but from these initial results consider that it is of potential benefit to smaller-scale reintroduction or augmentation translocations. It is less potentially suited to broad acre restoration or where it could impact the parent locations. Further research could also investigate the quantities of home soil required to produce positive outcomes; our study conservatively used 50% home soil however much smaller quantities may be equally beneficial as inoculation medium. If this were the case, this technique could then be considered in larger scale translocations using small quantities of home soil 'inoculant'.

While Provenance treatment plants performed similarly to those from other treatments overall, the benefits gained by improved plant condition during propagation suggests this is an approach worth considering. In addition to positive outcomes for the plants themselves, reducing the costs associated with nutrient addition would be beneficial for conservation projects which are often community-driven and without recurrent funding. This study demonstrates that a nursery propagation culture can and should be incorporated into translocation planning design and that simple and cost-effective nursery practices are well placed as part of a translocation planning toolkit.

464 **References**

465 ACT. (2017). Conservator Guidelines for the Translocation of Native Flora and Fauna in the ACT. Environment,
466 Planning and Sustainable Development Directorate, ACT Government, Canberra
467 Barea J.M. and Jeffries P. (1995). Arbuscular mycorrhizas in sustainable soil-plant systems. Mycorrhiza. Springer,
468 pp. 521-560.
469 Bates D., Mächler M., Bolker B. and Walker S. (2015). Fitting Linear Mixed-Effects Models Using lme4. 2015 67:
470 48. https://doi.org/10.18637/jss.v067.i01
471 Batty A.L., Dixon K.W., Brundrett M. and Sivasithamparam K. (2001). Constraints to symbiotic germination of
472 terrestrial orchid seed in a mediterranean bushland. New Phytologist 152: 511-520.
473 https://doi.org/10.1046/j.0028-646X.2001.00277.x.
474 Baum C., El-Tohamy W. and Gruda N. (2015). Increasing the productivity and product quality of vegetable crops
475 using arbuscular mycorrhizal fungi: A review. Scientia Horticulturae 187: 131-141.
476 https://doi.org/10.1016/j.scienta.2015.03.002.
477 BOM. (2019). Climate statistics for Australian locations Pennant Hills (Yarrara Road) monitoring station.
478 Australian Bureau of Meteorology. http://www.bom.gov.au.
479 Bothe H., Turnau K. and Regvar M. (2010). The potential role of arbuscular mycorrhizal fungi in protecting
480 endangered plants and habitats. Mycorrhiza 20: 445-457. https://doi.org/10.1007/s00572-010-0332-4.
481 Brancaleoni L., Gerdol R., Abeli T., Corli A., Rossi G. and Orsenigo S. (2018). Nursery pre-treatment positively
482 affects reintroduced plant performance via plant pre-conditioning, but not via maternal effects. Aquatic
483 Conservation: Marine and Freshwater Ecosystems 28: 641-650. https://doi.org/10.1002/aqc.2888.
484 Brundrett M. and Abbott L. (1991). Roots of Jarrah Forest Plants .I. Mycorrhizal Associations of Shrubs and
485 Herbaceous Plants. Australian Journal of Botany 39: 445-457. https://doi.org/10.1071/BT9910445.
486 Brundrett M., Bougher N., Dell B., Grove T. and Malajczuk N. (1996). Examining mycorrhizal associations.
487 Working with mycorrhizas in forestry and agriculture. Australian Centre for International Agricultural Research
488 Canberra, pp. 179-183.
489 Casselman C.N., Fox T.R., Burger J.A., Jones A.T. and Galbraith J.M. (2006). Effects of silvicultural treatments on
490 survival and growth of trees planted on reclaimed mine lands in the Appalachians. Forest Ecology and
491 Management 223: 403-414. https://doi.org/10.1016/j.foreco.2005.12.020.
492 Chialva M., Salvioli di Fossalunga A., Daghino S., Ghignone S., Bagnaresi P., Chiapello M., Novero M., Spadaro D.,
493 Perotto S. and Bonfante P. (2018). Native soils with their microbiotas elicit a state of alert in tomato plants. New
494 Phytologist 220: 1296-1308. https://doi.org/10.1111/nph.15014.
495 Cochrane A., Kelly A., Brown K. and Cunneen S. (2002). Relationships between seed germination requirements
496 and ecophysiological characteristics aid the recovery of threatened native plant species in Western Australia.
497 Ecological Management & Restoration 3: 47-60. https://doi.org/10.1046/j.1442-8903.2002.00089.x.
498 Commander L.E., Coates, D.J.,Broadhurst, L. ,Offord, C.A., Makinson, R.O. and Matthes M. (2018). Guidelines for
499 the Translocation of Threatened Plants Third Ed. Australian Network for Plant Conservation Inc
500 Cuesta B., Villar-Salvador P., Puértolas J., Jacobs D.F. and Benayas J.M.R. (2010). Why do large, nitrogen rich
501 seedlings better resist stressful transplanting conditions? A physiological analysis in two functionally contrasting
502 Mediterranean forest species. Forest Ecology and Management 260: 71-78.
503 https://doi.org/10.1016/j.foreco.2010.04.002.
504 Cuneo P., Emery N., Errington G. and Sherieff A. (2018). Assisted run(a)way: Translocation planning to secure
505 the Bankstown Hibbertia. Australasian Plant Conservation: Journal of the Australian Network for Plant
506 Conservation 27: 23-25.
507 Dalrymple S.E., Banks E., Stewart G.B. and Pullin A.S. (2012). A meta-analysis of threatened plant reintroductions
508 from across the globe. Plant Reintroduction in a Changing Climate. Springer, pp. 31-50.
509 DPIE. (2020). Julian's Hibbertia - profile. NSW Department of Planning, Industry and Envrionment.
510 https://www.environment.nsw.gov.au/threatenedSpeciesApp/profile.aspx?id=20279.
511 Duponnois R. and Plenchette C. (2003). A mycorrhiza helper bacterium enhances ectomycorrhizal and
512 endomycorrhizal symbiosis of Australian Acacia species. Mycorrhiza 13: 85-91. https://doi.org/10.1007/s00572-
513 002-0204-7.
514 Falk D.A., Millar C.I. and Olwell M. (1996). Restoring diversity: strategies for reintroduction of endangered
515 plants. Island Press, Washington, DC.
516 Fernández M., Marcos C., Tapias R., Ruiz F. and López G. (2007). Nursery fertilisation affects the frost-tolerance
517 and plant quality of Eucalyptus globulus Labill. cuttings. Annals of Forest Science 64: 865-873.
518 https://doi.org/10.1051/forest:2007071.

519 Fisher J.B. and Jayachandran K. (2002). Arbuscular mycorrhizal fungi enhance seedling growth in two
520 endangered plant species from South Florida. International Journal of Plant Sciences 163: 559-566.
521 https://doi.org/10.1086/340428.
522 Franco J.A., Martínez-Sánchez J.J., Fernández J.A. and Bañón S. (2006). Selection and nursery production of
523 ornamental plants for landscaping and xerogardening in semi-arid environments. The Journal of Horticultural
524 Science and Biotechnology 81: 3-17. https://doi.org/10.1080/14620316.2006.11512022
525 Gemma J., Koske R. and Habte M. (2002). Mycorrhizal dependency of some endemic and endangered Hawaiian
526 plant species. American Journal of Botany 89: 337-345. https://doi.org/10.3732/ajb.89.2.337.
527 Godefroid S., Le Pajolec S. and Van Rossum F. (2016). Pre-translocation considerations in rare plant
528 reintroductions: implications for designing protocols. Plant ecology 217: 169-182.
529 https://doi.org/10.1007/s11258-015-0526-0.
530 Godefroid S., Piazza C., Rossi G., Buord S., Stevens A.-D., Aguraiuja R., Cowell C., Weekley C.W., Vogg G. and
531 Iriondo J.M. (2011). How successful are plant species reintroductions? Biological Conservation 144: 672-682.
532 https://doi.org/10.1016/j.biocon.2010.10.003.
533 Grose M. (1989). Phosphorus nutrition of seedlings of the waratah, Telopea speciosissima (Sm) R.
534 Br.(Proteaceae). Australian Journal of Botany 37: 313-320. https://doi.org/10.1071/BT9890313.
535 Grossnickle S.C. (2012). Why seedlings survive: influence of plant attributes. New Forests 43: 711-738.
536 https://doi.org/10.1007/s11056-012-9336-6.
537 Groves R. and Keraitis K. (1976). Survival and growth of seedlings of three sclerophyll species at high levels of
538 phosphorus and nitrogen. Australian Journal of Botany 24: 681-690. https://doi.org/10.1071/BT9760681.
539 Guerrant Jr E.O. (2013). The value and propriety of reintroduction as a conservation tool for rare plants. Botany
540 91: v-x. https://doi.org/10.1139/cjb-2012-0239.
541 Guerrant Jr E.O. and Kaye T.N. (2007). Reintroduction of rare and endangered plants: common factors,
542 questions and approaches. Australian Journal of Botany 55: 362-370. https://doi.org/10.1071/BT06033.
543 Hadley Wickham R.F., Henry L. and Müller K. (2020). dplyr: A Grammar of Data Manipulation. R package version
544 0.8. 5. https://CRAN.R-project.org/package=dplyr.
545 Handreck K.A. (1997). Phosphorus requirements of Australian native plants. Australian Journal of Soil Research
546 35: 241-289. https://doi.org/10.1071/S96060.
547 Handreck K.A., Black N.D. and Black N. (2002). Growing media for ornamental plants and turf 3rd Ed. UNSW
548 Press Ltd.
549 Haskins K.E. and Pence V. (2012). Transitioning plants to new environments: beneficial applications of soil
550 microbes. In: Maschinski J. and Haskins K. E. (eds), Plant Reintroduction in a Changing Climate. Island Press,
551 Washington, DC., pp. 89-107.
552 Hoffmann W.A. and Poorter H. (2002). Avoiding Bias in Calculations of Relative Growth Rate. Annals of Botany
553 90: 37-42. https://doi.org/10.1093/aob/mcf140.
554 Hothorn T., Bretz F. and Westfall P. (2008). Simultaneous Inference in General Parametric Models. Biometrical
555 Journal 50: 346-363. https://doi.org/10.1002/bimj.200810425.
556 IUCN S. (2013). Guidelines for reintroductions and other conservation translocations. Gland Switz Camb UK
557 IUCNSSC Re-Introd Spec Group.
558 Jacobs D.F. and Landis T.D. (2009). Hardening [Chapter 12]. In: Dumroese R., Kasten; Luna, Tara; Landis and D T.
559 (eds), Nursery manual for native plants: A guide for tribal nurseries-Volume 1: Nursery management. Agriculture
560 Handbook 730, US Department of Agriculture, Forest Service, Washington, DC, pp. 217-227.
561 Jacobs D.F., Oliet J.A., Aronson J., Bolte A., Bullock J.M., Donoso P.J., Landhäusser S.M., Madsen P., Peng S., Rey-
562 Benayas J.M. and Weber J.C. (2015). Restoring forests: What constitutes success in the twenty-first century?
563 New Forests 46: 601-614. https://doi.org/10.1007/s11056-015-9513-5.
564 Keith D. (2006). Ocean Shores to Desert Dunes: The Native Vegetation of New South Walcs and thc ACT. NSW
565 Dcpartmcnt of Environmcnt and Conscrvation, Sydney.
566 Kuznetsova A., Brockhoff P.B. and Christensen R.H.B. (2017). lmerTest package: tests in linear mixed effects
567 models. Journal of Statistical Software 82. https://doi.org/10.18637/jss.v082.i13.
568 Lawrence B.A. and Kaye T.N. (2008). Direct and indirect effects of host plants: implications for reintroduction of
569 an endangered hemiparasitic plant (Castilleja levisecta). Madroño 55: 151-159. https://doi.org/10.3120/0024-
570 9637(2008)55[151:DAIEOH]2.0.CO;2.
571 Leake S. (1993). Phosphorus and Iron Nutrition in Australian Native Plants. Australian Native Plant Society.
572 http://www.anpsa.org.au/APOL1/mar96-2.html.
573 Maschinski J. and Albrecht M.A. (2017). Center for Plant Conservation's Best Practice Guidelines for the
574 reintroduction of rare plants. Plant diversity 39: 390-395. https://doi.org/10.1016/j.pld.2017.09.006.

Mendes R., Garbeva P. and Raaijmakers J.M. (2013). The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiology Reviews 37: 634-663. 10.1111/1574-6976.12028.
Menges E.S. (2008). Restoration demography and genetics of plants: when is a translocation successful? %J Australian Journal of Botany. 56: 187-196. https://doi.org/10.1071/BT07173.
Menges E.S., Smith S.A. and Weekley C.W. (2016). Adaptive introductions: how multiple experiments and comparisons to wild populations provide insights into requirements for long-term introduction success of an endangered shrub. Plant diversity 38: 238-246. https://doi.org/10.1016/j.pld.2016.09.004.
Michaelis J. and Diekmann M. (2018). Effects of soil types and bacteria inoculum on the cultivation and reintroduction success of rare plant species. Plant ecology 219: 441-453. https://doi.org/10.1007/s11258-018-0807-5.
Monks L., Coates D., Bell T. and Bowles M.L. (2012). Determining success criteria for reintroductions of threatened long-lived plants. Plant Reintroduction in a Changing Climate. Springer, pp. 189-208.
Montalvo A.M. and Ellstrand N.C. (2000). Transplantation of the subshrub Lotus scoparius: testing the home-site advantage hypothesis. Conservation Biology 14: 1034-1045. https://doi.org/10.1046/j.1523-1739.2000.99250.x.
Oliet J.A., Puértolas J., Planelles R. and Jacobs D.F. (2013). Nutrient loading of forest tree seedlings to promote stress resistance and field performance: a Mediterranean perspective. New Forests 44: 649-669. https://doi.org/10.1007/s11056-013-9382-8.
Oliet J.A., Tejada M., Salifu K.F., Collazos A. and Jacobs D.F. (2009). Performance and nutrient dynamics of holm oak (Quercus ilex L.) seedlings in relation to nursery nutrient loading and post-transplant fertility. European Journal of Forest Research 128: 253-263. https://doi.org/10.1007/s10342-009-0261-y.
Paul-Victor C., Züst T., Rees M., Kliebenstein D.J. and Turnbull L.A. (2010). A new method for measuring relative growth rate can uncover the costs of defensive compounds in Arabidopsis thaliana. New Phytologist 187: 1102-1111. https://doi.org/10.1111/j.1469-8137.2010.03325.x.
Price C. and Munns R. (1999). Chapter 6 - Growth analysis: a quantitative approach. In: Atwell B. J., Kriedemann P. E. and Turnbull C. G. (eds), Plants in action: adaptation in nature, performance in cultivation. Macmillan Education AU.
Reiter N., Lawrie A. and Walsh N. (2013). The mycorrhizal associations of Borya mirabilis, an endangered Australian native plant. Muelleria 31: 81-88.
Reiter N., Lawrie A.C. and Linde C.C. (2018). Matching symbiotic associations of an endangered orchid to habitat to improve conservation outcomes. Annals of Botany 122: 947-959. https://doi.org/10.1093/aob/mcy094.
Reiter N., Vlcek K., O'Brien N., Gibson M., Pitts D., Brown G.R., Bower C.C. and Phillips R.D. (2017). Pollinator rarity limits reintroduction sites in an endangered sexually deceptive orchid (Caladenia hastata): implications for plants with specialized pollination systems. Botanical Journal of the Linnean Society 184: 122-136. https://doi.org/10.1093/botlinnean/box017.
Rokich D.P. and Dixon K.W. (2007). Recent advances in restoration ecology, with a focus on the Banksia woodland and the smoke germination tool. Australian Journal of Botany 55: 375-389. https://doi.org/10.1071/BT06108.
Ruisi-Besares P. (2019). Inter-situ Restoration andthe Use of Whole Soil Inocula to Rehabilitate the Critically Endangered Hawaiian Plant, Cyrtandra kaulantha (Master's Thesis). University of Hawai'i at Manoa
Salifu K.F. and Jacobs D.F. (2006). Characterizing fertility targets and multi-element interactions in nursery culture of Quercus rubra seedlings. Annals of Forest Science 63: 231-237. https://doi.org/10.1051/forest:2006001.
Salifu K.F., Jacobs D.F. and Birge Z.K. (2009). Nursery nitrogen loading improves field performance of bareroot oak seedlings planted on abandoned mine lands. Restoration Ecology 17: 339-349. https://doi.org/10.1111/j.1526-100X.2008.00373.x.
Schott K.M., Snively A.E., Landhäusser S.M. and Pinno B.D. (2016). Nutrient loaded seedlings reduce the need for field fertilization and vegetation management on boreal forest reclamation sites. New Forests 47: 393-410. https://doi.org/10.1007/s11056-015-9522-4.
Shapcott A., Olsen M. and Lamont R.W. (2009). The importance of genetic considerations for planning translocations of the rare coastal heath species Boronia rivularis (Rutaceae) in Queensland. Ecological Restoration 27: 47-57. https://doi.org/10.3368/er.27.1.47.
Silcock J. (2018). Plants Red Hot List: Australia's 100 most endangered plants. Science for Saving Species Research findings factsheet Project 2.4. Threatened Species Recovery Hub, National Environmental Science Program. https://tsrhub.worldsecuresystems.com/.

630 Silcock J.L., Simmons C.L., Monks L., Dillon R., Reiter N., Jusaitis M., Vesk P.A., Byrne M. and Coates D.J. (2019).
631 Threatened plant translocation in Australia: A review. Biological Conservation 236: 211-222.
632 https://doi.org/10.1016/j.biocon.2019.05.002.
633 Specht R., Connor D. and Clifford H. (1977). The heath-savannah problem: the effect of fertilizer on sand-heath
634 vegetation of North Stradbroke Island, Queensland. Australian Journal of Ecology 2: 179-186.
635 https://doi.org/10.1111/j.1442-9993.1977.tb01135.x.
636 Standish R., Stokes B., Tibbett M. and Hobbs R. (2007). Seedling response to phosphate addition and inoculation
637 with arbuscular mycorrhizas and the implications for old-field restoration in Western Australia. Environmental
638 and Experimental Botany 61: 58-65. https://doi.org/10.1016/j.envexpbot.2007.03.004.
639 Stoneman G., Dell B. and Turner N. (1995). Growth of Eucalyptus marginata (jarrah) seedlings in mediterranean-
640 climate forest in south-west Australia in response to overstorey, site and fertiliser application. Forest Ecology
641 and Management 79: 173-184. https://doi.org/10.1016/0378-1127(95)03608-3.
642 Team R.D.C. (2019). R: A language and environment for statistical computing. R Foundation for Statistical
643 Computing, Vienna, Austri. https://www.R-project.org/.
644 Thomson V. and Leishman M. (2004). Survival of native plants of Hawkesbury Sandstone communities with
645 additional nutrients: effect of plant age and habitat. Australian Journal of Botany 52: 141-147.
646 https://doi.org/10.1071/BT03047.
647 Timmer V.R. (1997). Exponential nutrient loading: a new fertilization technique to improve seedling
648 performance on competitive sites. New Forests 13: 279-299. https://doi.org/10.1023/a:1006502830067.
649 Trubat R., Cortina J. and Vilagrosa A. (2010). Nursery fertilization affects seedling traits but not field
650 performance in Quercus suber L. Journal of arid environments 74: 491-497.
651 https://doi.org/10.1016/j.jaridenv.2009.10.007.
652 Trubat R., Cortina J. and Vilagrosa A. (2011). Nutrient deprivation improves field performance of woody
653 seedlings in a degraded semi-arid shrubland. Ecological engineering 37: 1164-1173.
654 https://doi.org/10.1016/j.ecoleng.2011.02.015.
655 Van Rossum F. and Raspé O. (2018). Contribution of genetics for implementing population translocation of the
656 threatened Arnica montana. Conservation genetics 19: 1185-1198. https://doi.org/10.1007/s10592-018-1087-2.
657 Wardle D.A., Bardgett R.D., Klironomos J.N., Setälä H., Van Der Putten W.H. and Wall D.H. (2004). Ecological
658 linkages between aboveground and belowground biota. Science 304: 1629-1633.
659 https://doi.org/10.1126/science.1094875
660 Zimmer H.C. (2016). Limits to recruitment of a rare conifer: Wollemia nobilis (PhD Thesis).
661 http://hdl.handle.net/11343/58611.
662 Zubek S., Turnau K., Tsimilli-Michael M. and Strasser R.J. (2009). Response of endangered plant species to
663 inoculation with arbuscular mycorrhizal fungi and soil bacteria. Mycorrhiza 19: 113-123.
664 https://doi.org/10.1007/s00572-008-0209-y

665

666 **Tables**

667 **Table 1** Mean nutrients (±SE) for growing medium treatments (High, Low, Provenance) measured
668 after 5 months in propagation. All values are in mg/kg, except C which is %. Home represents the
669 mean nutrients for soil from the five field planting sites. Provenance refers to the standard nursery
670 potting medium, mixed with 50% home soil (i.e., Provenance mix). Different lower-case letters
671 indicate significant differences between treatment levels (treatment contrasts, $P < 0.05$)

Treatment	High	Low	Provenance	Home
NH_4	275 (47.0) [a]	50.4 (44.6) [b]	25.2 (63.0) [b]	9.7 (0.8)
$N0_3$	258.3 (42.17) [a]	202.9 (40.5) [ab]	76 (52.7) [b]	0.79 (0.1)
P	79.7 (12.3) [a]	46.8 (11.7) [ab]	27.4 (16.6) [b]	6.75 (0.2)
K	412.9 (60.8) [a]	227.6 (48.2) [b]	169.4 (68.2) [b]	80.6 (5.3)
C	4.5 (0.09) [a]	4.8 (0.09) [b]	4.6 (0.12) [ab]	1.9 (0.1)

672

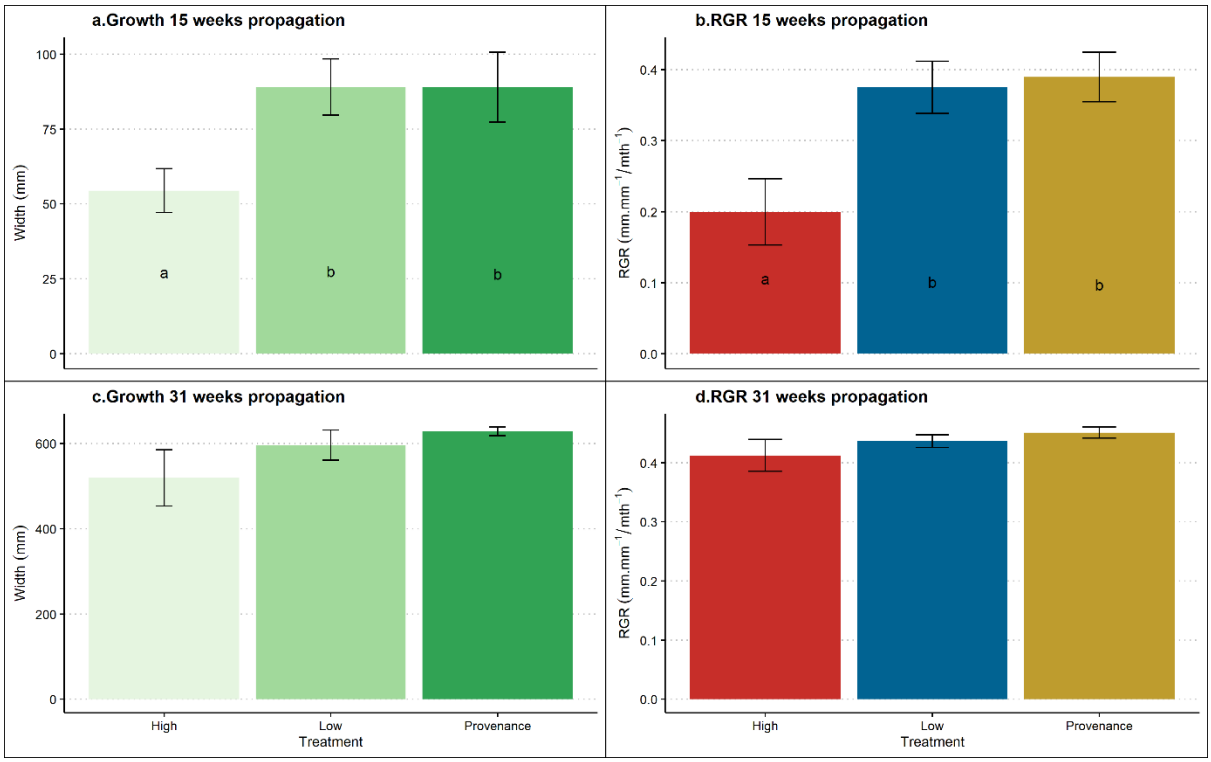
673 **Figure captions**

674 **Fig 1** Growth (width mean ± SE) of *H. spanantha* after 15 weeks (top left) and 31 weeks (7 months)
675 propagation (top right), plus relative growth rate width (RGR) (mean ± SE) of *H. spanantha* after 15
676 weeks (bottom left) and 31 weeks (bottom right) measured in mm. mm-1/month-1. For consistency
677 between propagation and planting measurements, growth (height) and RGR (height) is discussed in
678 text. Treatments are not significant, except where specified. Different letters indicate significant
679 differences between means (treatment contrasts, $P < 0.05$)

680 **Fig 2** Growth (width mean ± SE) of *H. spanantha* field-planted after 15 weeks (top left) and 47 weeks
681 (11 months) post-planting (top right). Treatments are not significant

682 **Fig 3** Flowering (peak flower count mean ± SE) of *H. spanantha* post planting among pre-planting
683 nutrient loading. Different letters indicate significant differences between means (Tukey contrasts, P
684 < 0.05)

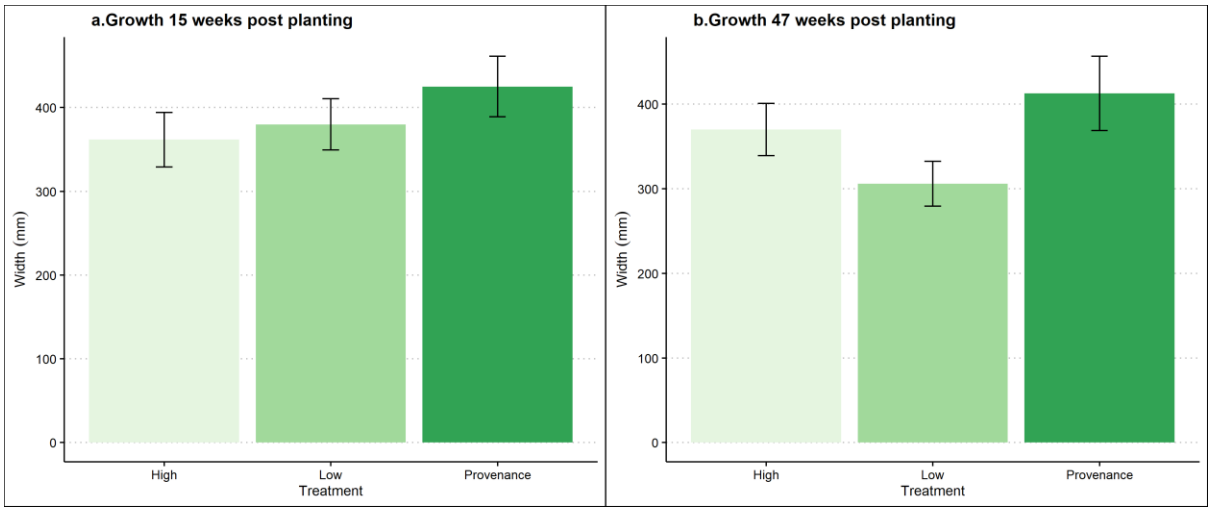
685 Figure 1.



686

687

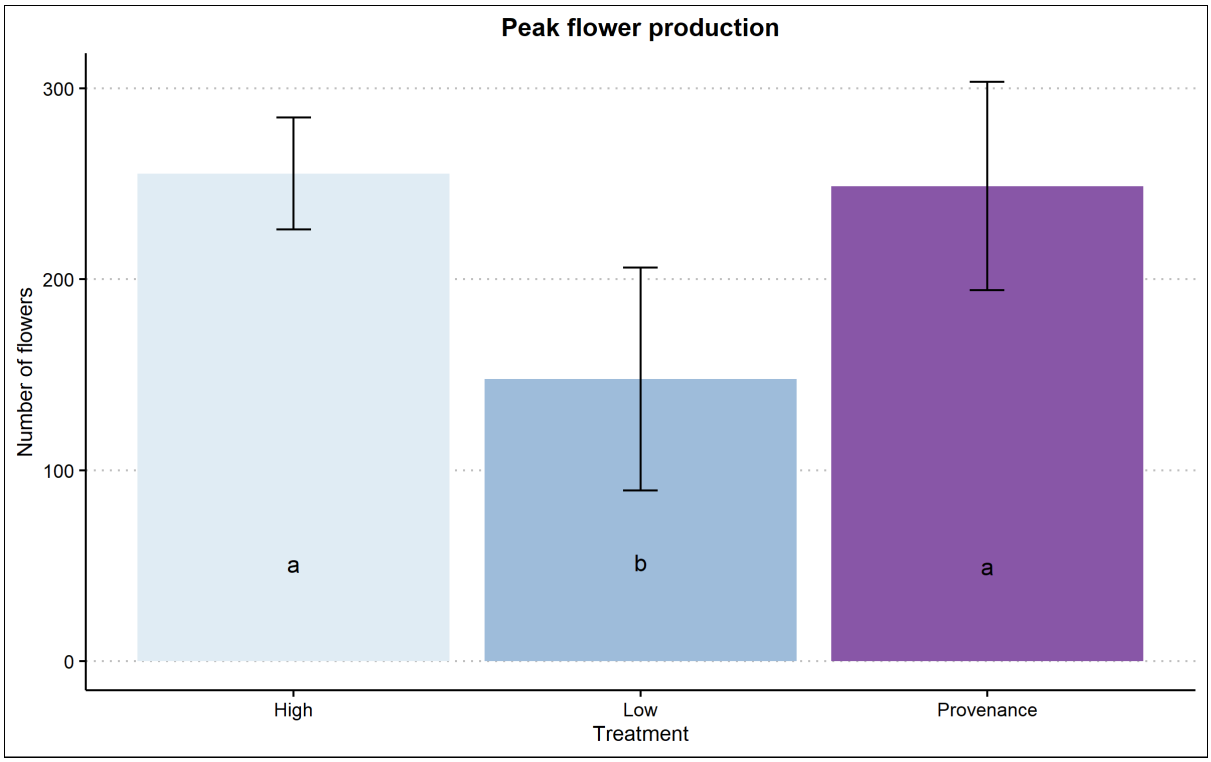
688 Figure 2.



689

690

691 Figure 3.



692

693

694 **Supplementary material captions (for accessibility)**

695 **Supplementary material 1** Image of flowering adult *H. spanantha* subshrub. Credit CDoyle

696 **Supplementary material 2**

697 **Figure S1** Indicative images of plants under High, Low and Provenance treatment at 10- and 21-
698 weeks propagation showing size and chlorotic growth. Credit CDoyle

699 **Table S1** Chlorotic severity scores for plants from each nutrient treatment level (mean ±SE) at 15 and
700 27 weeks. Different lower-case letters indicate significant differences between severity (Dunn
701 Kruskal-Wallis multiple comparison, $P < 0.05$)

702 **Table S2** Mean nutrients (±SE) in soils at each planting location. All values are in mg/kg except C
703 which is %. Different lower-case letters indicate significant differences between locations (treatment
704 contrasts, $P < 0.05$)

705 **Table S3.** Linear mixed effects models for (a) plant growth in the field at 47 weeks and (b) peak
706 flowering in response to propagation treatments of High nutrient mix, Low nutrient mix and
707 Provenance mix. For the fixed effects, reference level is Provenance. Significant values (< 0.05) are
708 highlighted in bold.

709

710

711

712