*A charity fostering scientific research into the biology and cultivation of the Australian flora*

# Research Matters

Newsletter of the Australian Flora Foundation

## No. 37, January 2023

Inside

# President's Report 2022

Delivered by Assoc. Prof. Charles Morris at the Annual General Meeting, November 2022

The 2022 granting round has seen a substantial increase in research expenditure that is now possible because of the Reed Bequest. Applications were invited under a two-tier program of larger grants (up to $60K) and smaller grants (up to $20K). The larger grants have been termed 'Malcolm Reed Grants' to honour the role of Malcolm in enabling these grants. The two tables below set out the successful applicants under the two granting programs, for total expenditure of $257K).

## Malcolm Reed Large Grants

| Principal investigator | Project title | Funding approved ($) |
|---|---|---|
| R Gleadow | Role of nitrogen-fixing cyanobacteria in growth and toxicity of culturally significant *Cycas* | 33,750 |
| E Gunn | To move or not to move, that is the question: understanding climate adaption to inform management options | 59,848 |
| JY Yap | Securing representative generic diversity within seed collections of two critically endangered species, *Rhodamnia rubescens* and *Rhodomyrtus psidioides* | 57,281 |
| G Liyanage | Deciphering dormancy, germination and storage requirements for *Zieria* | 59,682 |
| **Total** | | **210,561** |

## Small Grants

| Principal investigator | Project title | Funding approved ($) |
|---|---|---|
| E Tudor | Local adaptation as a driver for intraspecific variation in climate resilience of *Stylidium* | 13,353 |
| R Binks | Assessing genetic diversity, translocation success and future management options for the Critically Endangered *Grevillea acropogon* | 18,326 |
| N Anderson | Threatening processes and the future of mature Red Tingle trees (*Eucalyptus jacksonii*) | 15,154 |
| **Total** | | **46,833** |

Final reports were received from A Shapcott (Diversity and conservation of central Queensland rainforest), E Tsen (Historic gene flow and demographics of *Ryparosa kurrangi*), A Satyanti (Germination and seedling survival of Australian alpine flora) and S Schmidt (Functional response of *Triodia*).

Thanks are also due to the hard working members of Council who keep the granting program and administration of the Foundation going. A very special thanks must go to Peter Goodwin, who is retiring from the position of Grants Officer, which he has held since 2006. Peter also served as President during this period, for a number of years. Thanks, Peter, for your unstinting dedication and contribution to the running of the Council and administration of the Grants scheme. Professor Hans Griesser is taking over the role of Grants Officer. Ian Cox is a very capable and competent Secretary; Michelle Leishman heads the Scientific Committee, Tina Bell organizes the excellent Newsletter, and Jennifer Firn oversees the Foundation’s web page. Thanks are due to Council members, ordinary members and our donors, all of whom allow the Foundation to function and support plant research.

E Charles Morris
President
21 November 2022

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## Australian Flora Foundation Grants awarded

Seven grants were awarded by the Foundation for research to begin in December 2022.

### Role of nitrogen-fixing cyanobacteria in growth and toxicity of culturally significant *Cycas*

R Gleadow and Georgia Lloyd
Monash University, VIC

This project aims to document the contribution that cyanobacteria make to the nitrogen economy of *Cycas*, adding value to study of cycad toxicity and the place of cycads in Australian Indigenous cultures. Cycads produce nitrogen-containing neurotoxins that are harmful to humans unless processed. It is hypothesised that toxicity correlates with nutrient supply. *Cycas* form symbioses with nitrogen-fixing cyanobacteria inside specialised roots. Anatomical studies will determine the location and abundance of cyanobacteria. Reliance on fixed nitrogen for neurotoxin synthesis will be measured using stable nitrogen isotopes. This complements Indigenous knowledge and may lead to improved cultivation and conservation.

## To move or not to move, that is the question: understanding climate adaption to inform management options

Ellen Gunn[1], Peter Harrison[1], Julianne O'Reilly-Wapstra[1], and Dean Williams[2]
[1]University of Tasmania, TAS; [2]Sustainable Timber Tasmania, TAS

This project aims to evaluate the extent to which plant populations are adapted to future climates and elucidate the capacity of plants to respond plastically to climate stress. We expect to generate new knowledge on the genetic and epigenetic processes underlying plant adaptation and tolerance to climate extremes using innovative next generation sequencing approaches coupled with novel techniques to measure physiological responses. Results will enhance the conservation of plant gene pools, refine strategies to build resilience in restored populations, and triage the need for assisted migration in the face of climate change to ensure long-term survival of Australia’s iconic flora.

## Securing representative generic diversity within seed collections of two critically endangered species, *Rhodamnia rubescens* and *Rhodomyrtus psidioides*

Jia-Yee Samantha Yap[1], Jason Bragg[1], Stephanie Chen[1], Veronica Viler[1], and Julie Percival[2]
[1]Royal Botanic Gardens & Domain Trust, NSW; [2]Booderee National Park, NSW

*Rhodomyrtus psidioides*, also known as Native Guava, is a small tree that was once distributed widely in rainforest habitats of eastern Australia. It has undergone steep decline due to its high susceptibility to Myrtle Rust, a disease caused by the fungal pathogen, *Austropuccinia psidii*. The pathogen is wind dispersed, and rust spores have spread and infected the plants across its distribution. With no effective control currently available, susceptible species such as *R. psidioides* and *Rhodamnia rubescens* (Brush Turpentine) are assumed to be on a trajectory towards extinction.

Likely outcomes from this project, based on methodologies applicable to other species impacted by Myrtle Rust, are:

1. Enhanced understanding of genetic diversity within existing seed collections
2. Identification of mating patterns under nursery conditions
3. Optimisation of spatial arrangement of plants in *ex-situ* collections to generate genetically diverse seed collections

This project aims to improve knowledge on current seed collections, learn more about mating patterns of *R. rubescens* under nursery conditions and test the efficacy of a strategic placement of *ex-situ* plants results in a production of seed that replaces wild seed collection.

## Deciphering dormancy, germination, and storage requirements for *Zieria*

[1]Ganesha Borala Liyanage, [1]Karen Sommerville, [1]Cathy Offord, and [2]Mark Ooi
[1]Royal Botanic Gardens and Domain Trust, NSW; [2]University of New South Wales, NSW

This project aims to investigate seed germination and *ex situ* storage requirements for common *Zieria* species and extrapolate the findings to threatened *Zieria* species. The genus *Zieria* is in the Rutaceae family and contains 60 species, 59 of which are endemic to Australia. Many species have highly restricted distributions and have been identified as priorities for conservation. *Zieria* occupies a range of habitats, including rainforests, rainforest margins, fire prone ecosystems, wet and dry sclerophyll forests, vine thickets, exposed coastal headlands, rock outcrops and woodlands. Despite their occurrence in a wide range of habitat types, 20 of 39 *Zieria* taxa occurring in New South Wales have been identified as priorities for conservation under the 'Saving Our Species' project. As with other threatened plants found in NSW, changed environmental conditions, clearing of habitats and other disturbances associated with development have been, and continue to be, the main threats for genus *Zieria*. *Ex situ* conservation, and the production of seedlings for restoration, requires knowledge of how to germinate and successfully store seeds, data that is lacking for many *Zieria* species.

## Local adaptation as a driver for intraspecific variation in climate resilience of *Stylidium*

[1]Emily Tudor, [1]Erik Veneklaas, [2]Wolfgang Lewandrowski, and [2]Siegfried Krauss
[1]The University of Western Australia, WA; [2]Department of Biodiversity, Conservation and Attractions, WA

Intraspecific variation in stress tolerance can minimise local extinctions. However, the cascading effects of abiotic stress during different developmental stages on population fitness are often overlooked. Broadly, this research will examine the impacts of temperature and water availability on the physiological performance of *Stylidium hispidum* across key developmental stages (germination, growth, and flowering) and explore the consequences to reproductive success. Combined with the broader objectives of a PhD research project, the funding requested will support research that delivers an ontogenetically-explicit understanding of the drivers of species persistence and tolerance to rapid environmental change.

## Assessing genetic diversity, translocation success and future management options for the Critically Endangered *Grevillea acropogon*

[1]Rachel Binks, [1]Leonie Monks, and [2]Rachel Standish
[1]Department of Biodiversity, Conservation and Attractions, WA; [2]Murdoch University, WA

This project aims to implement outstanding recovery actions for *Grevillea acropogon*. In doing so, the project will expand on the current ecological knowledge and generate new genomic data for the species to investigate genetic diversity across the species and assess the success of previous seed collections and translocation activities. These data will provide an updated understanding of the current conservation status of the species, provide recommendations for priority conservation activities still needed for species recovery, and inform the most appropriate means of undertaking such activities. The project will also produce immediate conservation outcomes through the provision of seed to the Western Australian Seed Centre-Kensington and the use of all seedlings grown for the project in a translocation.

## Threatening processes and the future of mature Red Tingle trees (*Eucalyptus jacksonii*)

[1]Nate Anderson, [1]Nik Callow, [1]Alison Lullfitz, [2]Ryan Tangney, [3]Joe Fontaine, and [4]Grant Wardell-Johnson
[1]The University of Western Australia, WA; [2]University of New South Wales, NSW; [3]Murdoch University, WA; [4]Curtin University, WA

Forests in south western Australia are vulnerable to ecosystem transition or collapse due to the impacts of climate change and altered fire regimes. *Eucalyptus jacksonii* (Red Tingle) are narrow-range endemic forest giants confined to the wettest portion of the state and therefore likely to be highly vulnerable to water stress while also requiring careful fire management. Our work will investigate the vulnerability of mature Red Tingles to both altered fire regimes and a warming/drying climate, providing insight for tall forest management in a changing climate.

Top left: Native Guava (*Rhodomyrtus psidioides*), image from Atlas of Living Australia (ALA) provided by JW Wrigley, License CC-BY 3.0 (Au). Top right: Red Tingle (*Eucalyptus jacksonii*), image from ALA provided by Larivera, License CC-BY-NC 4.0 (Int). Bottom left: *Grevillea acropogon*, image from ALA provided by M Fagg, License CC-BY 3.0 (Au). Bottom right: White Butterfly Triggerplant (*Stylidium hispidum*), image from ALA provided by M Lythe, License CC-BY-NC 4.0 (Int).

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## Young Scientist Awards

The Australian Flora Foundation awards prizes annually to encourage young scientists to continue studying the flora of Australia.

At the annual conference of the Ecological Society of Australia (ESA), held in late November-early December 2022, the Foundation's prizes were presented to the following two students.

**Outstanding spoken presentation on the biology or cultivation of an Australian plant**

Flowers and diversity over lawn: quantifying what people want to see in urban plantings
Ali Babington
Murdoch University, WA

## Abstract

Urban landscapes are a unique amalgamation of ecological and social influences. Successful urban plantings must be suited to local environmental conditions and be socially acceptable. Here, we investigate the nexus between environmental requirements and social preferences to achieve sustainable urban plantings, in the context of the Woody Meadow Project. This urban greening project uses diverse mixtures of Australian shrubs, adapted to local conditions, that are densely planted and coppiced (cut to 100–200mm above ground level) every few years. This creates long-lasting plantings that require minimal maintenance and generate a diversity of flowers for aesthetic appeal. We investigated public preferences for various plant and planting features associated with woody meadows in Perth, Western Australia. An online survey asked respondents (n = 1088) to rate and rank a series of computer-generated images of a roadside urban woody meadow that systematically changed planting arrangement and plant features. Preliminary analysis indicates 88.8% of respondents preferred images with full vegetation (with an understorey, mid, and canopy layer) and flowers. In comparison, 86.7% of respondents indicated a low preference for lawn. I will present further findings and discuss how these results can inform woody meadow design, and urban plantings more broadly, to connect emotion and ecology in urban landscapes. This will ensure future plantings can meet both social and ecological requirements, to improve the sustainability and liveability of our urban landscapes.


Manipulated images created by Ali and used in a survey to determine preferences for different Woody Meadow types in an urban setting. Top left: 'a' represents least preferred, bottom right: 'f' represents most preferred. Image courtesy of Ali Babington.

## About Ali

Ali is a PhD student at Murdoch University investigating social and environmental aspects of urban greening. Prior to embarking on a PhD, she completed a Master of Urban Horticulture at the University of Melbourne. She is passionate about Western Australian plants and how they can be used in urban areas for conservation, to create a sense of place, and to engage the public.


AFF Spoken Presentation winner for 2022, Ali Babington. Left: planting a woody meadow in East Cannington, top right: receiving her award at ESA, bottom right: Ali with her poster presentation at ESA. Images courtesy of Ali Babington.

### **Outstanding poster presentation on the biology or cultivation of an Australian plant**

Trait-mediated community assembly during experimental grassland restoration is altered by planting year rainfall
Joe Atkinson
University of New South Wales, NSW

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## The importance of fire in replenishing *Banksia conferta* populations

Stephen AJ Bell*
Conservation Science Research Group, School of Environmental and Life Sciences, College of Engineering, Science and Environment, The University of Newcastle, NSW

### Introduction

Understanding the ecology of threatened species is necessary for their effective long-term management, and appreciation of the role of fire and pollinator networks are key management objectives. However, identifying the balance between managing habitat for pollinators and other co-habiting species while also ensuring optimal fire regimes can be problematic. For example, the removal of fire disturbance from habitats in which plant species require this for vital life stages can expedite population decline and result in local extinction, while high frequency fire events may exhaust seed reserves or progressively weaken plants and similarly cause species loss. Fire impacts on habitat for pollinators can also be dramatic, resulting in temporal shifts in fauna populations while recovery occurs, followed by a return to the appropriate structural habitat conditions required for pollinators.

*Banksia conferta* is a serotinous shrub or small tree. In New South Wales (NSW) it is listed as Critically Endangered and occurs only in Coorabakh National Park, but the species is also present in the Lamington Plateau, Mt Barney, and Glasshouse Mountains regions of south-eastern Queensland. Following fire events, NSW populations are known to resprout from a plate-like lignotuber which can develop into a bulbous mass beneath the ground surface. Flowering occurs over winter and, just prior to anthesis, a strong musky 'wet towel' odour is present, becoming sweeter at anthesis suggestive of a mammalian attractant. While Eastern Pygmy Possums are thought to play an important pollinating role in Queensland populations, there is little data on the fauna actively pollinating NSW populations of the species.

In a recently published study on this species (Bell et al., 2022), we set out to determine why recruitment in *Banksia conferta* was poor by examining the structural composition of the NSW populations, the fire history of areas supporting the species, and investigating faunal visitors to inflorescences over a 6-month period. The NSW populations occur in former timber production forests, and the working hypothesis was that the relatively young age of these forests (with few habitat hollows) was unable to sustain a viable population of mammalian pollinators attracted to the flowers at night. We set out an array of camera traps between June and December 2019 within three populations of *Banksia conferta*, focused on specific inflorescences (not yet fully opened at commencement) and set to trigger by day and by night. The three study populations varied in their fire histories (11, 56 and 63 years since the last fire), and this was reflected in both the age and structure of populations of *B. conferta*. The highest density of individuals and the greatest proportion of young plants occurred in the most recently burnt population, and the best follicle production was also found in this population.

*Banksia conferta*. Left: Fully opened inflorescence; right: example of regenerating population of *B*. *conferta*, 11-years post-fire. Images courtesy of Stephen Bell.

## Pollinators detected

Six mammal and 10 bird species were captured using camera traps interacting with plants of *B*. *conferta*, with 10 species seen to probe inflorescences. Sugar Gliders and White-cheeked Honeyeaters combined comprised 66% of all fauna interactions (n = 691), and of all instances where *Banksia* inflorescences were probed (n = 400), 45% were by White-cheeked Honeyeaters and 18% by Sugar Gliders. Six fauna species (1 mammal, 5 birds) interacted with inflorescences but did not probe flowers. The highest activity was seen at the most recently burnt site (11 years post-fire: 188 bird and 75 mammal probes), with lower activity at the other two sites (5 years post-fire: 77 birds, 77 mammals; 63 years post-fire: 0 birds, 18 mammals).

Mammal species caught on camera interacting with *Banksia conferta* flowers. Top left: Sugar Glider; bottom left: Feather-tail Glider. Right: an example of camera traps used in this study. Images courtesy of Stephen Bell.

The three populations studied differed in their main fauna foragers. White-cheeked Honeyeaters clearly dominated captures and flower probes at the recently burnt site, but this species was virtually non-existent at the other two sites (two probes in total). Two species (Sugar Glider and Eastern Spinebill) comprised the bulk of all flower probes at the 56-year post-fire site, and this site also supported the greatest diversity of fauna foragers (9 species). Only two species were detected at the 63-year post-fire site, with Sugar Glider comprising almost all interactions, but no birds were recorded.

Birds commonly found to be interacting with flowers of *Banksia conferta*. Left: White-cheeked Honeyeater; right: Eastern Spinebill. Images courtesy of Stephen Bell.

## Implications

The hypothesis that an absence or low density of arboreal mammals within flowering *Banksia* stands was limiting pollination opportunities and hence new recruitment was not supported in this study. Two of four postulated pollinating mammals (Sugar Glider and Feather-tail Glider) were detected at all three sites investigated. Sugar Gliders were dominant at one site, while this species (and Eastern Spinebill) was equally prevalent at another. Additionally, Bush Rats, Common Ringtail Possums and Brown Antechinus were (rarely) captured visiting inflorescences. Five bird species (White-cheeked Honeyeater, Eastern Spinebill, Lewin's Honeyeater, Red Wattlebird, and Regent Bowerbird) were observed probing inflorescences, with White-cheeked Honeyeaters predominated in the youngest post-fire and only population consistently producing infructescences.

Very few instances of invertebrates foraging on flowers were captured by cameras, with ants and moths noted on less than 10 occasions, However, cameras were not set to target this fauna group and their role in pollination remains to be determined. Given the range of faunal visitors, *B. conferta* is likely to be a pollinator-generalist, with birds (and particularly White-cheeked Honeyeater) operating as the key species during the day and small mammals (particularly Sugar Glider) at night. Morphological features of pollen presenters and styles of *B. conferta* support vertebrate pollination (George, 1981). In this case, rigid styles are a trait adapted to vertebrate pollination, particularly for forcing pollen entry into the pollination chamber via the stigmatic groove.

Interestingly, Eastern Spinebills foraged predominantly in the oldest population (56 years post-fire) whereas White-cheeked Honeyeaters were rare. At this location, this species dominated diurnal visitation to *B. conferta*, but nocturnal visitation by Sugar Gliders was equally important. Eastern Spinebills are low in the hierarchy of foraging guilds, and other studies have inferred that Spinebills (Eastern and Western) avoid feeding in stands of *Banksia* dominated by larger honeyeaters. The presence of a resident population of White-cheeked Honeyeaters at the best flowering population of *B. conferta* is likely to have competitively excluded extensive foraging by Eastern Spinebills. Resource availability may also drive pollinators differences given that the density of *Banksia* plants in the 11-year post-fire population (7,060 plants $ha^{-1}$) was substantially greater than present at the other two older populations (1,460-1,480 plants $ha^{-1}$). This represents a considerable contrast in nectar resource availability, and it is possible that insufficient nectar at two of the study populations may preclude a resident population of White-cheeked Honeyeaters persisting in these areas.

## The influence of fire

Fire history and how it influences stand structure, floristic composition, and habitat was a substantial variable among the sites examined during this study. The only population supporting abundant follicles was burnt twice in recorded history: 63 and 11 years ago. In contrast, the long fire-free intervals of the other two populations (56 and 63 years ago) supported individual plants that were of considerable age. Time since fire and its influence on flowering and fruiting can differ for resprouting and fire-sensitive *Banksia* species and, for *B. conferta*, time to first flowering at the 11-year post-fire population was observed to be 8 years, and possibly sooner.

Fire plays a major role in the ecology of seed release in many *Banksia* species. Queensland populations of *B. conferta* are reportedly serotinous (George, 1981), releasing seeds from the canopy only after a fire event. The very few seedlings and numerous closed follicles observed in our study populations over recent years suggests that these populations also display this trait. Serotiny can be variable within a species and reflect environmental conditions, but nevertheless it compromises persistence in some species when fire is excluded from a population for extended periods of time.

There is currently no data on the collective number of infructescences and viable seed store within the canopy of *B. conferta* populations, but it is plausible that many hundreds or thousands of seeds may be present.

Data from this study found a total of 665 old and current inflorescences on 30 plants across all three populations, but only 251 of these had follicles developed (no counts of viable seeds per infructescence were undertaken). Despite this, the absence of fire from two populations for over 55 years remains a concern, and low follicle production (despite the presence of pollinators) and decaying older follicles suggests that little new recruitment will occur without management intervention. Fortunately, the ability of this species to resprout from lignotubers and sub-surface roots should ensure persistence through drought and other stressors while awaiting fire.

Deriving appropriate fire prescriptions is difficult for many ecosystems and target species (Gosper et al., 2013), and these can vary across different landscapes. The results from this study suggest that a fire (prescribed or wildfire) in one or both long unburnt populations would be beneficial to rejuvenate habitat and promote germination of any viable seeds that remain in the canopy before further follicle decay occurs. Importantly, this should be followed by a fire-free interval of perhaps 10 years to allow plant maturation and restoration of the canopy seed store.

## References

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## *About the author

Dr Stephen Bell is a vegetation ecologist and consultant botanist, and is an Adjunct Lecturer at The University of Newcastle. He has been involved in native vegetation survey, classification and mapping in the Greater Sydney and Hunter Regions of New South Wales since 1990. His full profile can be viewed here:
https://www.newcastle.edu.au/profile/stephen-bell

# Propagation of therapeutic-honey *Leptospermum* plants by tissue culture, synthetic seeds, and cuttings

Ian Darby[A], Aaron Wiegand[A], Shahla Hosseini Bai[B], Helen Wallace[B], and Stephen Trueman[B]

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[B]Centre for Planetary Health and Food Security, Griffith University, QLD

## Introduction

Mānuka honey is harvested in New Zealand from hives of honeybees that forage on *Leptospermum scoparium* flowers (Morgan et al., 2019). The honey possesses antimicrobial and wound-healing properties because of its high methylglyoxal (MGO) concentration. The MGO is produced in ageing honey from dihydroxyacetone (DHA), which is found at high levels in the nectar of *L. scoparium* and a few other *Leptospermum* species (Williams et al., 2018). Demand for therapeutic honey greatly exceeds supply. This has driven the establishment of nectar plantations of species such as *L. polygalifolium* and *L. scoparium* that produce high amounts of DHA. *Leptospermum scoparium* occurs naturally in New Zealand and south-eastern Australia, while *L. polygalifolium* occurs along the east coast of Australia.

*Leptospermum* seed can be difficult to extract from the fruit or to germinate, which has slowed down plantation establishment. We therefore developed methods for propagating *L. polygalifolium* and *L. scoparium* by tissue culture, synthetic seeds, and cuttings. Tissue culture allows very rapid propagation of plants from a limited supply of starting material. Synthetic seeds are alginate-encapsulated mini-cuttings that combine the advantages of true-to-type propagation by tissue culture or cuttings with the convenient storage and handling of conventional seeds. Cutting propagation has the advantage of allowing plant production in a nursery without the need for laboratory facilities, although it can be slower than tissue culture.

## Tissue culture

We surface sterilised *L. polygalifolium* and *L. scoparium* seeds and germinated them *in vitro* to initiate tissue culture (Darby et al., 2021a). We then transferred the seedling shoots to full-strength Murashige and Skoog (MS) medium. The shoots were dissected into nodal explants after 5 weeks (Fig. 1a) and transferred to fresh medium with various concentrations of benzyladenine (BA). The shoots were maintained for 7 weeks, dissected again, and transferred to fresh medium with the same BA concentration for another 8 weeks. We repeated the same process for a final passage of 8 weeks.

Tissue culture of *L. polygalifolium* was successful using either 0 or 2.2 µM BA. New nodes were produced on elongating lateral shoots in the hormone-free medium (Fig. 1b). Each *L. polygalifolium* seed cultured using this medium produced a median of 584 shoots (range: 0-5,427) in 32 weeks. Multiple short shoots developed from callus in the medium containing BA (Fig. 1c). Each *L. polygalifolium* seed cultured using this medium produced a median of 630 shoots (range: 0-58,635) in the same timeframe. Tissue culture of *L. scoparium* was successful only in hormone-free medium. The median number of *L. scoparium* shoots produced from each seed was 659 (range: 0-5,985) in 32 weeks.

We induced roots by transferring the shoots to hormone-free half-strength MS medium and maintaining them for 1 week in darkness and 3 weeks in the light. We then transferred the plantlets (Fig. 1d) into propagation tubes containing eucalypt seedling mix (Darby et al., 2021a). The tubes were maintained in a sealed tub in the laboratory for 4 weeks and then transferred to a glasshouse propagation chamber. Plantlets were maintained within the tub under shade cloth for 3 days, before the tub lid was opened gradually and the light level increased over a further 12 days. This method was successful for root formation and nursery acclimatisation of 31% of *L. polygalifolium* shoots and 44% of *L. scoparium* shoots (Fig. 1e).

### Synthetic seeds

We also excised nodal explants to produce synthetic seeds from the shoots produced in tissue culture (Darby et al., 2022). The explants were cut to 4-5 mm length. They were placed in full-strength MS medium with 3% sodium alginate for 10 minutes. We then drew up each explant using a pipette with a 7-mm diameter opening and dispensed it into 100 mM calcium chloride solution. This caused the globule of encapsulation solution to form a skin that encapsulated the explant. We retrieved the synthetic seeds by decanting off the solution after 30 minutes (Fig. 2a).

We germinated the synthetic seeds successfully in hormone-free emergence medium containing full-strength MS. Synthetic seeds produced only shoots if they were placed on emergence medium that contained BA (Fig. 2b, c). Both shoot and root emergence (Fig. 1d) occurred from 26-57% of *L. polygalifolium* synthetic seeds and 100% of *L. scoparium* synthetic seeds that were placed on the hormone-free medium.

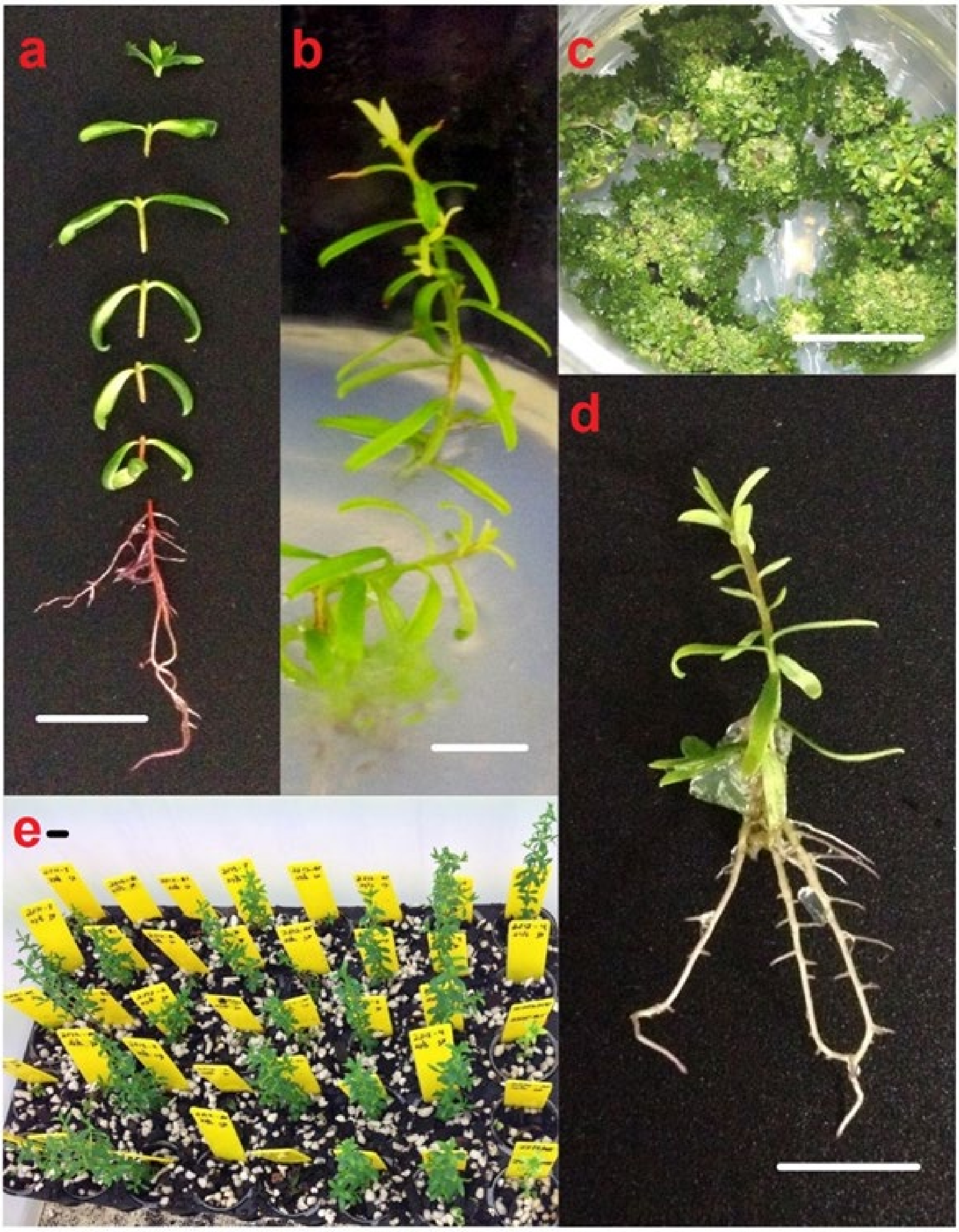


Figure 1. Tissue culture of *Leptospermum polygalifolium and L. scoparium*. (a) Seedling shoot dissected into nodal explants; (b) *L. polygalifolium* shoot with elongating lateral shoots in the absence of benzyladenine (BA); (c) *L. polygalifolium* producing multiple short shoots in medium with 2.22 μM BA; (d) *L. polygalifolium* plantlet with three adventitious roots; (e) *L. scoparium* plantlets acclimatised to the nursery. Scale bars = 2 cm. Images courtesy of Ian Darby.

Complete germination of *L. scoparium* synthetic seeds was surprising for a medium that did not contain hormones. Hormone-free medium was designed as a control treatment for what we anticipated would be ongoing research to develop a germination method. Instead, this simple medium proved 100% effective for germinating *L. scoparium* synthetic seeds. These synthetic seeds can be stored and transported easily between laboratories and nurseries. They might also have the capacity for direct transfer to plant nurseries (Hung and Trueman 2012; Hung and Dung, 2015).

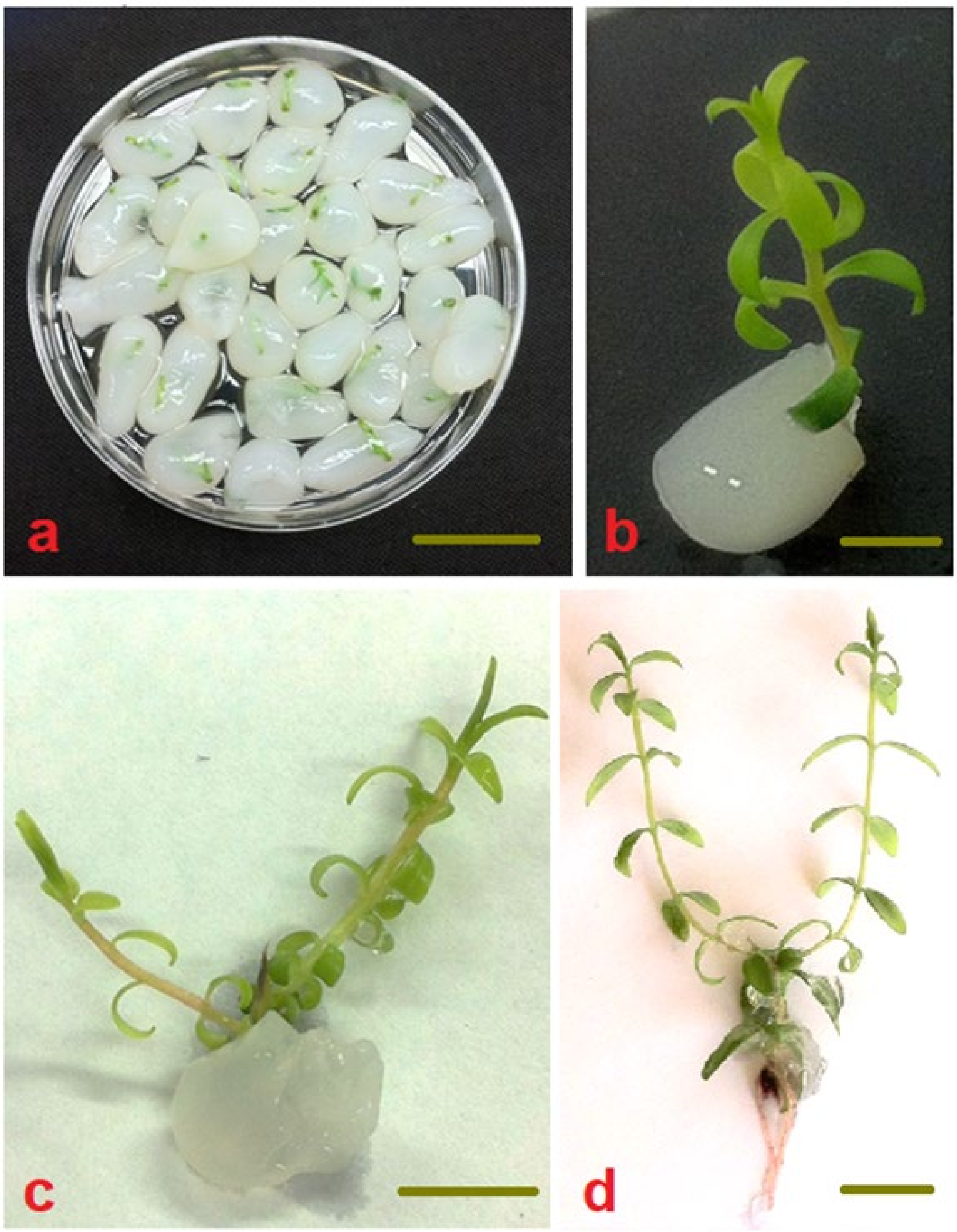


Figure 2. Synthetic seeds of *Leptospermum polygalifolium* and *L. scoparium*. (a) Freshly-made synthetic seeds of *L. polygalifolium*; (b) Synthetic seed of *L. polygalifolium* with one emerged shoot; (c) Synthetic seed of *L. polygalifolium* with two emerged shoots; (d) Synthetic seedling of *L. scoparium* with roots and shoots. Scale bars: (a) 10 mm, (b-d) 5 mm. Images courtesy of Ian Darby.

## Cuttings

We raised nursery stock plants by transplanting the plantlets produced from tissue culture into 1.1-L pots (Fig. 3a, b) that contained a eucalypt potting mix (Darby et al., 2021b). We maintained them as stock plants at 15 cm height by harvesting cuttings. Stock plants of *L. polygalifolium* each produced 104-144 cuttings over a 14-week period from transplanting in late spring until late summer. Stock plants of *L. scoparium* each produced 56-74 cuttings over the same period.


Figure 3. Stock plants of (a) *Leptospermum polygalifolium* and (b) *L. scoparium*; (c) cuttings of *L. scoparium* in propagation tubes; and (d) cutting of *L. polygalifolium* with roots. Scale bars: (a) 60 mm, (b) 55 mm, (c) 35 mm, (d) 20 mm. Images courtesy of Ian Darby.

We dipped the base of each cutting into talcum powder or a talcum-powder-based formulation containing indole-3-butyric acid (IBA), a commonly used rooting hormone. We placed each cutting into a tube containing eucalypt propagation mix (Darby et al., 2021b). The propagation tubes were then placed in the glasshouse propagation chamber (Fig. 3c). Most cuttings formed roots (Fig. 3d), with no effect of rooting hormone on the rooting percentages. Strike rates for cuttings without rooting hormones were 88-93% for *L. polygalifolium* and 65-76% for *L. scoparium*.

As a result, the production capacity over summer was 95-126 new plants from every stock plant of *L. polygalifolium* and 37-53 new plants from every stock plant of *L. scoparium*. This plant production capacity was greater than for many subtropical eucalypts used in forestry and horticulture (Darby et al., 2021b), mainly because of the high strike rates of *Leptospermum* cuttings.

## Conclusion

Therapeutic-honey *Leptospermum* plants are highly amenable to propagation through tissue culture, synthetic seeds or cuttings:

- Tissue culture has the capacity to produce many hundreds or thousands of *Leptospermum* plants from a single seed in less than a year.
- Synthetic seeds provide a convenient method for mass-propagating, storing, and distributing *Leptospermum* germplasm, potentially with capacity for direct transfer to nurseries.
- Cuttings are a low-technology, nursery-ready method for *Leptospermum* plant production. *Leptospermum* cuttings have very high strike rates, decreasing the wastage of potting mix and reducing the costs of sorting unrooted cuttings from newly formed plants.

Each of these propagation methods can be used, alone or in combination, to speed up plant production for high-value nectar plantations.

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# Australian Flora Foundation Final Report: Raising Rarity: testing the horticultural potential of rare and threatened Australian wildflowers

Sue Murphy, John Delpratt, and Megan Hirst
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**This information has been taken from a Final Report submitted to the AFF**

## Project summary

Conservation horticulture aims to retain genetic and phenotypic variation present in natural populations, while commercial production seeks a uniform product, often through clonal propagation or the use of highly selected seed. While the efficiencies of large-scale commercial horticultural production may seem an obvious way to save rare and threatened plants, the selection of plant material or propagules from a limited range of sources may further compromise at-risk species, especially if the impetus to conserve variability is not clearly identified. One solution is to enhance the profile of rare and threatened species by developing selected, commercially attractive forms that can be made available to commercial growers, while maintaining separate, genetically diverse populations for use in species conservation.

The Raising Rarity project seeks to raise awareness of the plight of our flora restricted to ecosystems that are severely threatened by habitat loss, fragmentation, and climate change by growing and displaying rare and threatened Victorian species in an accessible horticultural setting. Plants are grown by seed in containers and transplanted to the Research Garden located within the Australian Garden at Royal Botanic Gardens Victoria (RBGV) Cranbourne. We are working with rare species that we believe have the potential for use in horticulture.

## Impact

Volunteers have been instrumental to the success of the project, and it is through their work, the RBGV was awarded the Protection of the Environment Award for the Raising Rarity project at the 2019 Keep Victoria Beautiful Awards. Student volunteers from the University of Melbourne tirelessly measured plants on weekends and advised customers on the growing conditions of our potted stock at the Cranbourne Friends Plant Sale. Cranbourne horticultural staff provided advice and installation of the drip irrigation system and ongoing maintenance to ensure the project ran smoothly. David Roberts and Gemma Cotterell of the RBGV Melbourne horticultural staff planted out a selection of Raising Rarity stock across their living collections and monitored plant progress over an 18-month period.

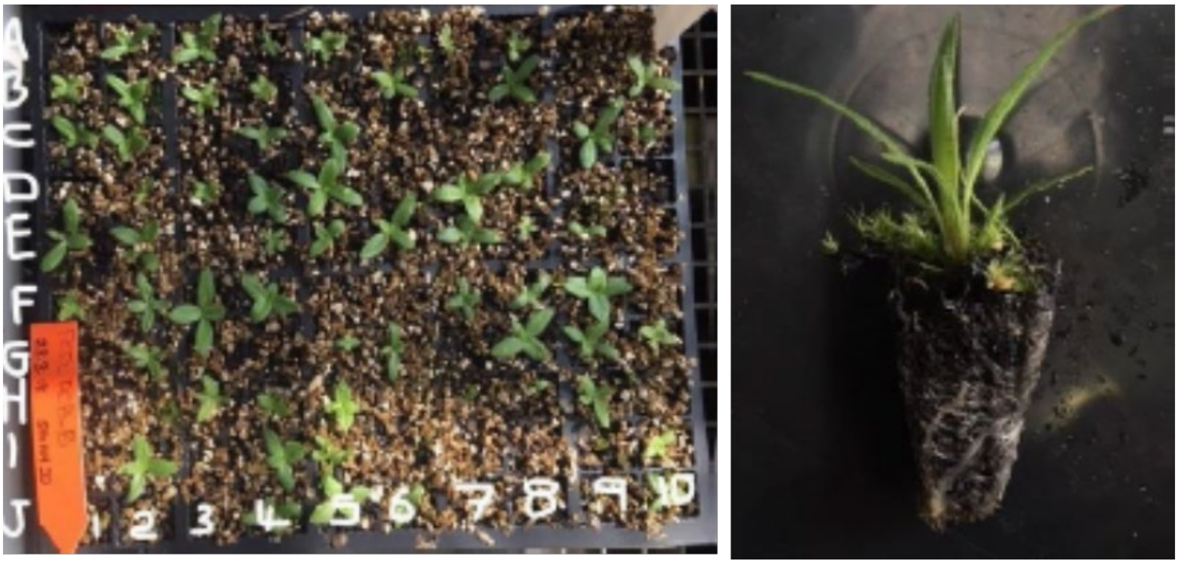
Top: Raising Rarity target species (left to right: *Podolepis muelleri*, *Brachyscome tadgellii*, *Podolepis laciniata*, *Rutidosis leptorhynchoides*, *Stylidium armeria* subsp. *pilosifolium*, *Glycine latrobeana*, *Leptorhynchos orientalis*, *Brachyscome chrysoglossa*, and *Leucochrysum albicans* subsp. *tricolor*) growing at The University of Melbourne – Burnley campus nursery. Images courtesy of MJ Hirst. Bottom left: Raising Rarity volunteers measuring plant growth in the outdoor research plots. Images courtesy of D. Jeetun. Bottom right: Getting Raising Rarity stock ready for sale at the Cranbourne Friends biannual plant sale. Images courtesy of B Jeffrey.

Throughout the Raising Rarity project there have been opportunities to learn from others working on plant selection and performance indicators as well as present our work to a greater audience. Due to the generosity of a BGANZ professional award scholarship, recipient Meg Hirst was able to travel to the Chicago Botanic Gardens in July 2018 to spend time with Mr. Richard Hawke, a Plant Evaluation and Associate Scientist.

## Seed, germination, and seedling assessment

Conventional seed testing was done to investigate the quality and viability of each seed collection. Across all species, seed fill was 90-100% and in most cases, seed had plump endosperm surrounding the embryo.

Plate tests for germination were done in sterile petri dishes containing 1% agar and, for species where dormancy or low germination was expected, gibberellic acid was used. Ten to 25 seeds were tested depending on availability. Plates were incubated in a germination cabinet on a 12-hour light: 12-hour dark photoperiod at 20°C and 15°C, respectively. Nursery sowings were undertaken in March 2018 with one seed per cell into 100 cell plastic trays with a pine bark-based sowing medium with a low-level controlled release fertiliser. Trays were placed in a glasshouse maintained at 18°C under natural light. Counts of seedling emergence were made weekly for up to 10 weeks. Seedlings were transferred from an individual cell into tubes when adequate root formation had occurred.




Left: Example of a 100-cell germination tray showing *Podolepis laciniata* seedlings in individual cells. Coding for each cell involves one letter per row and the accompanying number to provide unique identifier. Right: A seedling 'plug' of *Stylidium armeria* subsp. *pilosifolium* from an individual cell within a 100-cell germination tray. Images courtesy of M Hirst.

## Publications arising from this project

Hirst MJ, Messina A, Delpratt CJ, Murphy SM (2019) Raising rarity: Horticultural approaches to conserving Victoria's rare and threatened wildflowers. *Australasian Plant Conservation: Journal of the Australian Network for Plant Conservation*, 27, 14-16. https://search.informit.org/doi/10.3316/informit.431228558645109

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# Australian Flora Foundation Progress Report: Disentangling the genetic and environmental drivers of manna quality in *Eucalyptus viminalis* and its consequences for native ecosystem function

Erin Bok
University of Tasmania, TAS

**This information has been taken from a Progress Report submitted to the AFF**

## Project summary

The intentional movement of plant species to new habitats is a strategy that is being increasingly applied to mitigate the loss of biodiversity in the face of climate change. However, decisions are undertaken with little consideration of potential downstream impacts on ecosystem processes (e.g., resource provisioning). This project will investigate how genetics and the environment influences plant survival and resource provisioning (manna, a high-quality food resource) in a foundation tree species, *Eucalyptus viminalis*. Results will inform guidelines on the assisted movement of foundation tree species, providing the needed confidence in the environmental sector to make informed decisions to manage Australia's iconic forests.

## Background

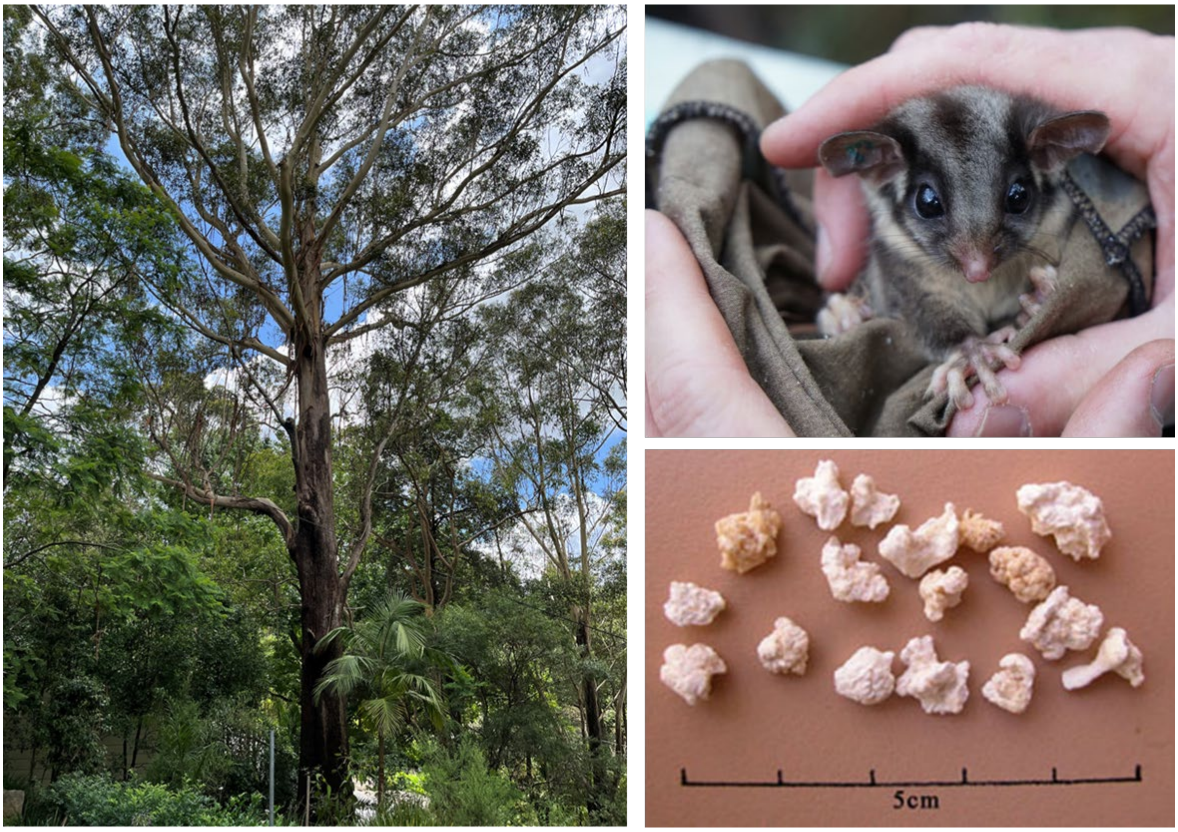
Manna is a sugary carbohydrate produced by *E. viminalis* that is a core food resource for honeyeaters (*Melithreptus* genus), insects (ants, beetles, butterflies, moths), gliders/possums (including the endangered Leadbeater's Possum) and the endangered Forty-spotted Pardalote (*Pardalotus quadragintus*). It is anticipated that the results from this research will provide key information to inform the targeted seed sourcing of forest tree species, such as *E. viminalis*, to establish climate-resilient and biodiverse ecosystems.

## Progress

A pilot study revealed that manna quality and production varied seasonally, suggesting that sampling should be conducted during late spring/summer to maximise likelihood of successfully extracting manna. Interestingly, this coincides with the peak breeding season of the Forty-spotted Pardalote which is endemic to Tasmania. This preliminary finding was used to schedule fieldwork for sampling of manna.

Three common garden sites were sampled to examine the extent of genetic by environment effects on manna production and quality. These sites, (Swansea, Liffey, and Coles Bay) were targeted as they maintained the highest survival and growth rates of *E. viminalis* and represented a gradient of aridity. The Swansea and Liffey sites fall at the driest and wettest ends of the aridity gradient, respectively, with Coles Bay between these two sites. A total of 300 individual trees have been sampled across these three sites. Three additional sites will be suitable for manna sampling in late spring/early summer 2023/24.

Variation in manna quality was high between all provenances, and within provenances across the three sites. This suggests there is both environmental and genetic variation in manna quality occurring. Early indications are that variation in manna from trees from wetter provenances is most likely underpinned by genetics rather than environment. Variation in manna production was more conserved and showed a general trend towards higher quantities of manna being produced by trees from more arid provenances.




Left: Example of mature Manna Gum (*Eucalyptus viminalis*) (image courtesy of Tina Bell). Top right: The iconic Leadbeater's Possum (*Gymnobelideus leadbeateri*), an arboreal mammal that feeds on manna (image from https://www.environment.vic.gov.au/conserving-threatened-species/threatened-species/lowland-leadbeaters-possum, image credit Zoos Victoria). Bottom right: Examples of solidified manna (original image from https://www.anbg.gov.au/apu/plants/eucavimi.html, original image credit Murray Fagg).

# Australian Flora Foundation Progress Report: How influential are plant neighbourhood interactions in community responses to extreme climatic events?

Melissa Gerwin
University of Tasmania, TAS

**This information has been taken from a Progress Report submitted to the AFF**

## Project Summary

This project focusses on how climate change will impact plant species interactions and, consequently, ecosystem function. The climate change experiment simulates three consecutive hot, dry summers in a factorial drought x warming x heatwave design. The design includes two levels of drought (0% and ~70% chronic drought), two levels of warming (ambient and warmed) and two levels of heatwave (no heatwave and heatwave). By mapping the fine-scale species composition in each treatment plot annually, we can determine the nature and strength of plant-plant interactions within this system presently and in future climates. This information on plant interaction outcomes (e.g., competition, facilitation) can then be connected to functional trait values, thus providing the mechanistic link between compositional patterns and functional processes that determine plant community stability. Additionally, if we find that plant functional traits are responding to climate manipulations, we will gain insight on how these trait changes might drive changes in community composition.

## Progress

All climatic treatments have been established. This involved building drought shelters and installing warming chambers over winter 2021. Later in the year, thermocouples, soil moisture sensors, and their accompanying infrastructure were installed and wired to data loggers. In summer 2021/2022, the footings for a 0.5 x 0.5 m survey grid were installed in each plot. The identity of all species within each grid square (2.5 x 2.5 cm) were recorded, creating a detailed compositional map of the control area. This compositional survey is in the process of being repeated for 2022/2023 and will be collected a third time in summer 2023/2024. The heatwave event for 2021/2022 was in accordance with climate projections for the area and involved heating plots for 2 days to ~35°C during daylight hours. Presently, preparations are underway for simulating the 2023 heatwave event.

Functional trait data for the seven most common forbs on site was collected during late 2022. This involved collecting three individuals of each species from each treatment plot (outside of fixed survey grid) and measuring plant characteristics such as height, number of leaves, and leaf length, width, and thickness. Leaf surface area, and fresh and dry weights were measured to calculate leaf area for calculation of specific leaf area and leaf dry matter content. Functional trait information for the most common grasses on site will be collected in early 2023. Additionally, carbon and nitrogen concentrations in the leaves will be measured in early 2023. These traits give insight into plant life history strategies and can be used to determine the mechanisms underlying plant interactions.

Top left and right: Drought shelters and warming chambers within the experiment. Bottom left: Wiring thermocouples and soil moisture sensors to datalogger, bottom right: Grid-frame secured into footings ready for compositional survey in a drought x warming plot. Images courtesy of Melissa Gerwin.

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## What Research Were We Funding 25 Years Ago?

**Note:** See http://aff.org.au/results/grant-summaries/ for further details of these and other research projects funded by the AFF.

In 1996 the AFF funded or contracted five research projects, three of which had a fire theme. This new direction of funding was in response to the 1994 bushfires in NSW. These fires burnt along the NSW coast from the Queensland border in the north to Batemans Bay in the south, and inland in the ranges in Greater Sydney, the Blue Mountains, and the

Central Coast. Tragically, four people died, 225 homes were destroyed, and 800,000 ha of bushland was burnt.

The Coronial inquest that followed resulted in the Rural Fire Service as it operates today. There were important improvements in equipment and training, the incorporation of new resources such as aircraft and long overdue improvements in legislation and policy. The AFF was associated with crucial basic fire research into fuel dynamics, fuel flammability and fire in rainforests. This work has informed many generations of students and researchers, including the author, over the past 25 years.

### Modelling interactions of fire and rainforest

John Crockett, Brendan Mackey, and Julian Ash
School of Botany and Zoology, Australian National University, ACT
Funded in 1996 for $10,470

This project was done to examine the potential importance of fire in governing the relative distribution of rainforest and sclerophyll forest, and the width and position of the boundary between them. A general fire model was used to examine factors important for determining fire behaviour in rainforest. There were consistent differences in the microclimates of the vegetation types studied – rainforest is more buffered from extremes in external conditions than sclerophyll forest or the boundary vegetation.

A litter moisture model was developed to examine the effect of altered microclimatic conditions and canopy cover on fuel drying. Microclimatic conditions in the rainforest cause leaf litter to retain moisture for longer than litter in the other vegetation types.

Surveying and experimentation in the laboratory focused on determining the possible effects of rainforest and boundary vegetation on fire spread. This work showed that differences in the nature of the live vegetation between rainforest, boundaries and sclerophyll forest directly contribute to the flammability of the vegetation. As such, rainforest and rainforest boundaries will burn when litter moisture is very low, however, the flammability of rainforest and rainforest boundaries remains well below those of sclerophyll forest even during extreme fire weather and fuel dryness conditions.

### Ignitibility of Australian native plants

Malcolm Gill and Peter Moore
CSIRO Division of Plant Industry, Canberra, ACT
Funded in 1996 for $24,700

The study found that the flammability of plants is a function of their intrinsic properties, the environment and other management practices.

The authors found that it is difficult to adequately describe the flammability of a species because of these factors and the effects of life stage (from seedling stage to senility). Even the description of the flammability of a single plant will have major problems for these reasons. Direct measurement is also fraught with difficulty. Despite these problems there are attributes of major importance to the determination of flammability (in broad terms) that may be considered in a semi-quantitative way. These features continue to pave the way for development of a flammability rating for vegetation around houses – gardens, parks, and native bush reserves.

### Changes in flammability of vegetation in relation to fire frequency: fuel dynamics after prescribed fire and wildfire in forests of the ACT

John Raison
CSIRO Division of Forestry, Canberra, ACT
Funded in 1996 for $13,000

The Australian Flora Foundation funded some of the research on the re-accumulation of litter and understorey biomass following prescribed fires in sub-alpine eucalypt forests in the ACT.

The pattern of change in fuel mass and fuel structure after fire is a major factor affecting development of fire risk, fire behaviour, and the impacts of fire on a range of ecological values.

Fine surface litter dynamics following low-intensity prescribed burns were quantified for sub-alpine eucalypt forests. In Alpine Ash (*Eucalyptus delegatensis*), Broad-leaved Peppermint (*E. dives*) and Snow Gum (*E. pauciflora*) forests, fine (<6 mm diameter components) litter re-accumulates rapidly after prescribed burning reaching a mass of 10-12 t $ha^{-1}$ within 4-5 years. Understorey vegetation can accumulate a further 3 t $ha^{-1}$ of combustible biomass in this time. The quasi steady-state mass of accumulated litter has been estimated for a range of forest types. This ranges from about 15 t $ha^{-1}$ in low altitude open forests to about 25 t $ha^{-1}$ in mature, high-altitude wet sclerophyll forests. The dynamics of another important fuel component – stringy and ribbon bark on tree trunks which form firebrands and result in spot fire development – is less well understood but is important for determining fire behaviour.

As examples of the ongoing influence of this research, the following three Honours projects recently completed at The University of Sydney, each used one or more of the final reports as sources as information or data. There will be many more examples from around the country.

*Fire and rain: The flammability of Australian rainforests*, Tallulah Dods
The study described the forest structure, composition, and leaf morphology of species in two types of rainforests and nearby wet

sclerophyll forests in the Nightcap National Park, NSW. All four vegetation types were found to differ in species composition and structure and, concomitantly, basic leaf morphology including leaf size, shape, and area. The two rainforests differed from each other as much as from their wet sclerophyll forest counterparts. Flammability of 14 representative species was ranked using Multicriteria Analysis and Principal Components Analysis.

*The morphology of plants and leaves form has a certain relationship with flammability*, Xinyue Zhan

Six published studies (including the one done by Gill and Moore) describing leaf flammability were examined to determine if there are commonly measured indicators of flammability. Across these studies, a total of 48 different variables were used to describe leaf morphology, chemistry, and flammability. Common variables were leaf length and width, leaf moisture content (equivalent to fuel moisture content and water content), and ignition delay time (equivalent to time to ignition). A recommendation for researchers to work towards a consensus for measurement of key set of indicators of flammability was made.

*Spatial and temporal variation in litterfall in eucalyptus forests*, Christy Hung

Litterfall in two dry sclerophyll forests in Victoria were examined over a period of 12 months. Differences in litter production indicated that litter production was influenced by landscape-scale factors such as location and less so than by fine-scale features such as topography or aspect. Coastal forests are strongly influenced by stochastic weather events (e.g., storms), whereas inland forests show expected seasonal patterns (e.g., high litterfall in summer).

Dry sclerophyll forests used for litterfall studies. Left: 'Inland' forest in Wombat State Forest, Victoria, right: 'coastal' forest in Orbost, Victoria. Images courtesy of Veronica Quintanilla.

# Financial Report

This statement is summarised from the Foundation's audited accounts for the year ended 30 June 2022

| | 2022 | 2021 |
|---|---|---|
| **Income** | **$** | **$** |
| Donations | 11,546 | 12,390 |
| Administration Contributions and Fees | - | 908 |
| Membership Subscriptions | 1,530 | 2,040 |
| Interest | 21 | 3,116 |
| Managed Fund Distributions | 346,765 | 74,535 |
| Sundry Income | 97 | - |
| Imputation Credits | 43,796 | 5,568 |
| Increase in Market Value of Investments | 43,889 | 145,558 |
| Bequest Reed Estate | 226 | 3,706,551 |
| Dividend CBA shares | 17,512 | - |
| **Total Income** | **465,382** | **3,950,666** |
| | | |
| **Expenses** | | |
| Grants | 62,129 | 53,438 |
| Decrease in Market Value of Investments | 1,647,097 | - |
| Audit Fees | 2,575 | 2,450 |
| Administration Expenses and Website | 727 | - |
| Postage and Printing | 547 | 186 |
| Young Scientist Awards | 1,000 | - |
| Australian Network for Plant Conservation | 1,500 | - |
| **Total Expenses** | **1,715,575** | **56,074** |
| | | |
| **Surplus (Deficit) for the Year** | **(1,250,193)** | **3,894,592** |
| | | |
| **Assets** | | |
| Investments and bank accounts | 3,326,553 | 4,808,325 |
| Debtors | 275,801 | 45,076 |
| Imputation Credits Receivable | 49,364 | 5,568 |
| GST Receivable | 2,652 | 5,445 |
| **Total Assets** | **3,654,370** | **4,864,414** |
| | | |
| **Liabilities** | | |
| GST Payable | 153 | 239 |
| Grant Commitments | 105,676 | 65, 441 |
| **Total Liabilities** | **105,829** | **65,680** |
| | | |
| **Net Assets** | **3,548,541** | **4,798,734** |
| | | |
| **Accumulated Funds** | | |
| Accumulated Funds from last year | 4,798,734 | 904,142 |
| Current Year Surplus (Deficit) | (1,250,193) | 3,894,592 |
| **Total Accumulated Funds** | **3,548,541** | **4,798,734** |

## About the Australian Flora Foundation

The Australian Flora Foundation is a not-for-profit charity dedicated to fostering scientific research into Australia's flora. It is totally independent. All members of the Council and the Scientific Committee give their time freely as volunteers.

Each year the Foundation provides funding for a number of grants for research into the biology and cultivation of the Australian flora. While the grants are not usually large, they are often vital in enabling such projects to be undertaken. Many of the researchers are honours or postgraduate students, and their success with an Australian Flora Foundation grant hopefully stimulates their interest in researching Australia's unique and diverse plants throughout their careers.

This work is only made possible by the generous support of donors and benefactors.



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