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

# *Research Matters*

Newsletter of the Australian Flora Foundation

*No. 39, January 2024*

Inside

# President's Report 2023

Delivered by Assoc. Prof. Charles Morris at the Annual General Meeting, November 2023.

The 2023 granting round continued the substantial increase in research expenditure that is now possible because of the Reed and other Bequests. Grant applications were invited under a two-tier program of larger grants (up to $60K; termed Reed Grants) and smaller grants (up to $20K). The two tables below set out the successful applicants under the two granting programs and more details of each project are provided later in the newsletter.

## Malcolm Reed Large Grants

| Principal investigator | Project title (abbreviated) | Funding approved ($) |
|---|---|---|
| Alfonzetti | Exploring seed microbiomes | 56,167 |
| Hodgins | Climate resilient grassland restoration | 55,812 |
| Hopley | Conservation of grevilleas | 59,928 |
| Wright | Critical seed sources for eucalypts | 60,000 |
| **Total** | | **231,907** |

## Small Grants

| Principal investigator | Project title (abbreviated) | Funding approved ($) |
|---|---|---|
| Cave | Investigation of infraspecific polyploidy | 19,730 |
| Kurosawa | Healthy carnivorous plants | 12,640 |
| Lawn | Conservation of montane grassland | 19,840 |
| **Total** | | **52,210** |

The following Final reports were received:

Diego Guevara, University of Adelaide, for the project '*The effects of soil microbial community and topsoil removal on grassland restoration techniques in South Australian mediterranean-type climate region*'. The project was approved for funding in 2020 and commenced in 2021.

Richard C. McLellan and David M. Watson, Charles Sturt University, for the project '*The status of populations of Australian Sandalwood (*Santalum spicatum*) in Australia's western rangelands*'. The project was approved for funding in 2020 and commenced in 2021.

Chris O'Brien, University of Queensland, for the project '*Cryo-biotechnology for conservation of endangered Sweet Myrtle (*Gossia fragrantissima *(F.Muell. ex Bently) N.Snow & Guymer)'*. The project was approved for funding in 2020 and commenced in 2021.

Marion Howard and Alison Shapcott, University of the Sunshine Coast, for the project '*Assessing the diversity and conservation of Central Queensland Coastal Rainforest using DNA barcoding*'. The project was approved for funding in 2017 and commenced in 2018.

Peri Tobias, University of Sydney, for the project '*Development of molecular markers for resistance to Myrtle Rust*'. The project was approved for funding in 2017 and commenced in 2018.

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 Michelle Leishman who is retiring from the Chair of the Scientific Committee. Thanks, Michelle, for your unstinting dedication and contribution to the running of the Grants scheme. Hans Griesser administers the Granting program as Grants Officer; Ian Cox is a very capable and competent Secretary; Jennifer Firn now heads the Scientific Committee; Tina Bell organises the excellent Newsletter. 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
31 October 2023

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## Australian Flora Foundation Grants awarded

Seven grants were awarded by the Foundation for research to begin in December 2023.

### Malcolm Reed Large Grants

Exploring seed microbiomes to develop bespoke microbial amendments for enhancing revegetation success of underutilised native species

Matthew Alfonzetti[1], Dr Sasha Tetu[1], Rachael Gallagher[2], and Marlien van der Merwe[3]
[1]Macquarie University, NSW; [2]Western Sydney University, NSW; [3]Botanic Gardens of Sydney, NSW

The proposed project aims to address seed storage constraints and low germination rates in ecosystem restoration efforts. It acknowledges the scarcity of viable native seeds, the cost and difficulty of seed procurement, and the limited supply for important species. Beneficial microbes on seed surfaces play important roles in germination and early-stage seedling growth, however, they are little-studied and rarely considered in revegetation. Current seed bank storage methods may lead to a loss of vital seed microbial diversity. This novel project will characterise the microbiomes of field-collected and stored seeds, compare their composition, and screen for plant-beneficial functions. The findings will guide the development of microbial amendments to support germination and growth of hard-to-germinate native species for restoration.

## Developing climate resilient grassland restoration strategies

Kathryn Hodgins[1], Rose Andrew[2], and Chris Lee[1]
[1]Monash University, VIC; [2]University of New England, NSW

In south-eastern Australia less than 1% of the original grasslands remain. Restoration is key to conservation of these threatened ecosystems, but climate change poses a significant additional threat. Our project aims to uncover the genetic basis of climate adaptation in two widespread grass species, *Bothriochloa macra* and *Bothriochloa decipiens*. We will use machine learning to predict genetic mismatches of current populations with respect to future climates and test these predictions using field experiments. We will couple this with genomically informed niche modelling to predict future distributions. Outcomes include a framework to optimise seed sourcing for grassland restoration, and genomic resources.

## Preserve and conserve – conservation of *Grevillea micrantha* and *Grevillea gariwerdensis*

Tara Hopley, Laura Simmons, and Russell Larke
Royal Botanic Gardens Victoria, VIC

Both *Grevillea gariwerdensis* and *Grevillea micrantha* have undergone significant reductions in population size and range, and without direct conservation action are at risk of further reductions and extinction. This project will utilise a fully integrated approach for the conservation of both species which will include, population genetic analysis, direct *in situ* management actions to mitigate risks, the establishment of genetically representative *ex situ* collections, and community involvement. Furthermore, nominating of *G. gariwerdensis* as a threatened species under the Flora and Fauna Guarantee Act 1988 (FFG) and the establishment of recovery plans for both species will provide both species with greater protection and inform future conservation initiatives.

## Identifying and conserving critical seed sources for climate ready restoration of Tasmania's eucalypt flora

Magali Wright[1], Peter Harrison[2], Rebecca Jones[2], and James Wood[3]
[1]Landscape Recovery Foundation, TAS; [2]University of Tasmania, TAS;
[3]Tasmanian Seed Conservation Centre, TAS

This project aims to evaluate the sensitivity and flexibility of a plant's germination response to rising temperatures and develop a transferable modelling framework to identify critical seed zones of conservation significance. We expect to generate new knowledge on the regenerative capacity of populations in the face of climate change and the potential of maternal effects to shift germination cues of offspring. Results will benefit the conservation of plant gene pools through targeted seed banking, refine strategies to build resilience in revegetated populations, and triage translocations within and outside a species range to ensure long-term survival of Australia's iconic flora.

Top left and right: Female and male flowers of *Bothriochloa macra*, a widespread species that will be used to uncover the genetic basis of climate adaptation of grasses (photos by L von Richter ©The Royal Botanic Gardens & Domain Trust available via PlantNet NSW). Bottom left: *Grevillea micrantha*, a small spreading shrub that is listed as "critically endangered" in Victoria (image licensed under the Creative Commons Attribution-Share Alike (CC BY-SA) 4.0 International license). Bottom right: *Grevillea gariwerdensis*, a species that was first formally described in 2000 from specimens collected near Halls Gap in 1966 (image licensed under the CC BY-SA 3.0 International license).

## Small Grants

### Investigation of the impact of infraspecific polyploidy on germination yields of *Eremophila* species

Robyn Cave[1], Lynn Hoffman[1], Hans Griesser[2], and Lyndal Thorburn[2]
[1]The University of Queensland, QLD; [2]Australian Native Plants Society (Australia)

The project objectives are to determine if ploidy affects seed germination and seedling vigour in *Eremophila* (Emu Bush) species, as observed in other genera. The project is significant because polyploid genotypes would require seed germination protocols that differ to diploid types but once germinated, they are likely to be more adaptable to climate change. The outcomes will assist land rehabilitation programs and the nursery industry with genotype selection and reduced establishment costs. The intended project benefits are to increase the diversity of Australian native plants used for land rehabilitation and ornamental purposes and to promote the conservation of *Eremophila* and its genetic diversity.

### Healthy carnivorous plants need a balanced diet: improving *Utricularia* conservation through refined diet estimates

Emmi Kurosawa and Joanne Oakes
Southern Cross University, QLD

Previously, we developed a new nitrogen stable isotope modelling approach and showed that Australian carnivorous bladderworts (in the genus *Utricularia*) decreased their carnivory with increased nutrients in their habitat. Our model has the potential to assess early wetland degradation and thereby contribute to conservation of *Utricularia*. However, the use of literature values for the trophic fractionation factor (TFF) adds uncertainty to our model. The TFF represents the change in isotope ratio during assimilation of food and has only been determined for animal consumers. In this project, for the first time, we will determine TFF for "botanical carnivory", allowing us to refine our model.

### Improving conservation of high value vegetation communities and rare plants in a nationally significant montane grassland

Pippi Lawn, James Kirkpatrick, and Vanessa Adams
University of Tasmania, TAS

Conservation of nationally significant montane grassland at the Vale of Belvoir, Tasmania, is challenged by a limited understanding of the patterns and processes influencing rare vegetation. The project aims to support management of priority native flora, by providing crucial information on: (a) vegetation in rare doline (sinkhole) features, a novel area of research within Australia; (b) a threatened vegetation community, highland *Poa* grassland; and (c) two threatened plant species, *Leucochrysum albicans* subsp. *tricolor* and *Stackhousia pulvinaris*. For *L*.

*albicans*, the project also tests the efficacy of management interventions to support recovery of the declining population of this endangered species.

Left: *Leucochrysum albicans* subsp. *tricolor*, a perennial everlasting daisy in the family Aseraceae (photo from https://www.threatenedspecieslink.tas.gov.au/Pages/Leucochrysum-albicans-subsp-tricolor.aspx). Right: *Stackhousia pulvinaris*, a perennial herb that forms dense mats to 10 cm high (photo by D Hardin ©The Royal Botanic Gardens & Domain Trust available via PlantNet NSW).

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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 Darwin in July 2023, the Foundation's prizes were presented to the following two students.

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

#### Closing the loop: recycled mineral products as sustainable substrates for native Australian urban plantings

Claire Kenefick
University of Melbourne, Victoria

#### Abstract

Greening urban landscapes benefits humans and nature. However, sustaining high-quality vegetation in public settings can be expensive resulting in the design of simple, low-quality landscapes. 'Naturalistic plantings' can improve landscape quality through careful species selection

for ecological and structural diversity, dense planting, and mineral substrates supporting plant growth and suppressing weeds. The mineral substrates are often mined materials such as sand or scoria. Mineral substrates recycled from construction and demolition wastes are more sustainable, though their ability to support plant growth in naturalistic plantings needs to be established.

We measured the physical and chemical properties of recycled mineral substrates – crushed concrete, crushed rock, recycled sand – compared to scoria. All substrates were alkaline and had good water-holding capacity (WHC), except for crushed rock which had high air-filled porosity (AFP). Pine bark mulch (PB) added at 10%, 20% and 50% v/volume altered physical and chemical properties with 50%, significantly lowering pH in all substrates, increasing WHC by 5% in crushed rock, and increasing AFP tenfold in recycled sand.

Following testing, we established a 3-month growth (biomass, leaf chlorosis and survival) experiment with two Australian shrubs, *Alyogyne huegelii* and *Goodenia ovata*, in substrate mixes with 10% and 50% PB. We hypothesised that substrates with 50% PB will have greater plant growth and survival, and that greater alkalinity in crushed concrete will cause lower biomass and greater leaf chlorosis. Identifying suitable recycled substrates for plant growth can improve sustainability and reduce cost which may increase uptake of naturalistic plantings across urban landscapes.

## About Claire

Claire is a PhD candidate at the University of Melbourne in the Green Infrastructure Research Group. Claire's experience as a geologist and environmental scientist has helped her transition to plant ecology focusing on plant-soil interactions in recycled substrates. Claire regularly posts updates on the progress of her research on Instagram: @claire_plants


Top left: Claire watering her first experiment after planting (March 2023). The experiment used two native shrub species (*Alyogyne huegelii* and *Goodenia ovata*) growing in four types of substrates (scoria, recycled sand, crushed rock, and crushed concrete) with addition of two amounts of pine bark (10% and 50%). Top right: Claire transporting a train of pots for her third experiment, which will be a trait analysis of the same 12 species as in experiment 2 to investigate if there are traits that help plants grow in highly alkaline crushed concrete and pine bark substrate mixes. Bottom: Claire after completion of planting of her second experiment using four different depths (5, 10 and 20 cm) of substrate mixes (made of 50% recycled concrete and 50% pine bark mulch) as growing media or 12 native shrub species.

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

### Large-scale patterns in delayed greening

Giancarlo Chiarenza
UNSW Sydney, Australia

Delayed greening is a mechanism in which plants do not deploy chlorophyll in young leaves until fully mature and thus often appear red, blue, or light green. Young leaves are not tough and thus suffer substantially more herbivory than mature leaves. Therefore, delaying the input of chlorophyll may reduce the loss of nutrients to herbivores. However, we have little quantitative evidence about cross-species and biogeographic patterns in delayed greening.

I sampled the most abundant ecosystems along a latitudinal gradient from Tasmania to northern Queensland, quantifying the amount of chlorophyll contained in expanding and mature leaves via spectroscopy. I quantified delayed greening as the log ratio of chlorophyll content of expanding and mature leaves. I found that delayed greening is not a binary state, nor does it have a bimodal distribution as previously thought, but it can be assimilated to a normal distribution. Delayed greening is considered primarily a tropical phenomenon, and while tropical species can show high levels of delayed greening, so do species from temperate ecosystems.

I theorize that in the tropics, plants with delayed greening, especially ones with striking red or blue juvenile leaves are more memorable, which led to the idea that delayed greening is found mostly there. While delayed greening is defined as an anti-herbivory defence, I did not find a statistical relationship between the amount of leaf damage and the level of delayed greening. I hypothesize that other factors, such as soil nutrients, might help us understand why young leaves are blushing while they’re flushing.

### About Giancarlo

Giancarlo is a plant ecologist fascinated in how plant-soil relationships can affect plant success and large-scale ecological patterns. He submitted his thesis in October 2023, and he is now looking to continue down the academic path as a Postdoctoral Fellow.

Top left: Giancarlo sampling trees during his PhD research. Top right: Juvenile leaves of *Gastrolobium spinosum* (Prickly Poison), bottom left: *Eucalyptus* sp., and bottom right: *Banksia oblongifolia* (Fern Leaf Banksia).

# The Australian brush-turkey: unwelcome guest or ecosystem engineer?

Matthew Hall and Dieter Hochuli

School of Life and Environmental Sciences, The University of Sydney, Sydney, New South Wales

## Introduction

If you asked a suburban resident of many parts of Sydney, Brisbane, Newcastle, the Gold Coast, Byron Bay, or any other city on the Australian East Coast north of Wollongong, it would seem like the country is in the midst of an invasion. The interloping offender is the Australian brush-turkey (*Alectura lathami*), a large native bird from the Megapode family, which has been spotted with increasing frequency in urban and suburban areas over the past few decades. Weighing up to 2.5 kg, brush-turkeys live naturally in the rainforests and woodlands of eastern Australia, from the Illawarra up to Cape York. There they can be found raking through the leaf litter for invertebrates, small lizards, seeds, fruit, and plant parts to eat. However, in the big city, this same behaviour is responsible for much of the ire these birds have drawn from residents, as brush-turkeys dig up carefully cultivated garden beds and lawns.

But their real impact on suburban gardens (and gardeners!) stems from their nest building behaviour. Unlike most birds, brush-turkeys do not brood their eggs. Instead, males construct huge mounds out of soil and leaf litter, often using multiple tonnes of material. These are natural incubators, keeping the eggs laid by the females warm through the heat produced by decomposition of the litter. Males must constantly maintain the mound to care for the eggs, adding and taking away material to keep the temperature just right for their offspring. This form of reproduction is unique among birds and is only practiced by the brush-turkey and the rest of the Megapode family. A brush-turkey mound may be an awe-inspiring sight in the bush, but it is often unwelcome in a carefully maintained suburban garden, which can be entirely raked bare to provide material for construction.

## A surprising suburban resident

While the spread of brush-turkeys in suburban areas is both a source of frustration for gardeners and novelty for birdwatchers, it is surprising from a scientific perspective. In the early 20$^{th}$ century, brush-turkeys were seen as exceedingly rare around cities and possibly even heading towards extinction. A combination of pressures from hunting, clearing of their native habitat, and predation from introduced species like cats and foxes, had led to sharp population declines. In 1867, the Sydney Mail newspaper reported "*a few years ago it was very plentiful in the Illawarra, Hunter River, and Clarence districts, but the continual persecutions of the*

*timber-cutters have made it very scare in those places*". However, this began to change in the second half of the 20th century after hunting was banned. Brush-turkeys began to slowly return to their former range, including now built-up but increasingly leafy areas of major cities such as Sydney and Brisbane.

Top left: Male brush-turkey; photo: M. Scott. Top right: Female brush-turkey; photo: M. Hall. Bottom left: Brush-turkeys foraging on the sidewalk in Mosman, Sydney; photo: J. Kelly. Bottom right: Brush-turkey mound in a suburban backyard; photo: A. Fitzgerald.

Brush-turkeys have been unexpectedly successful in cities. Their ground dwelling lifestyle, large and exposed ground nests, and lack of parental care for their chicks make them particularly vulnerable to the dangers of city life. Studies on radio tracked brush-turkeys found high mortality rates, with predation from feral cats making up a large proportion of fatalities. Further studies found that the species had lower reproductive success in cities and predicted that the spread of brush-turkeys in suburban areas would eventually slow. However, this slowdown never materialised, with brush-turkeys not only occupying more area over time, but also thriving in more built-up areas with less greenspace cover.

## Brush-turkeys as ecosystem engineers?

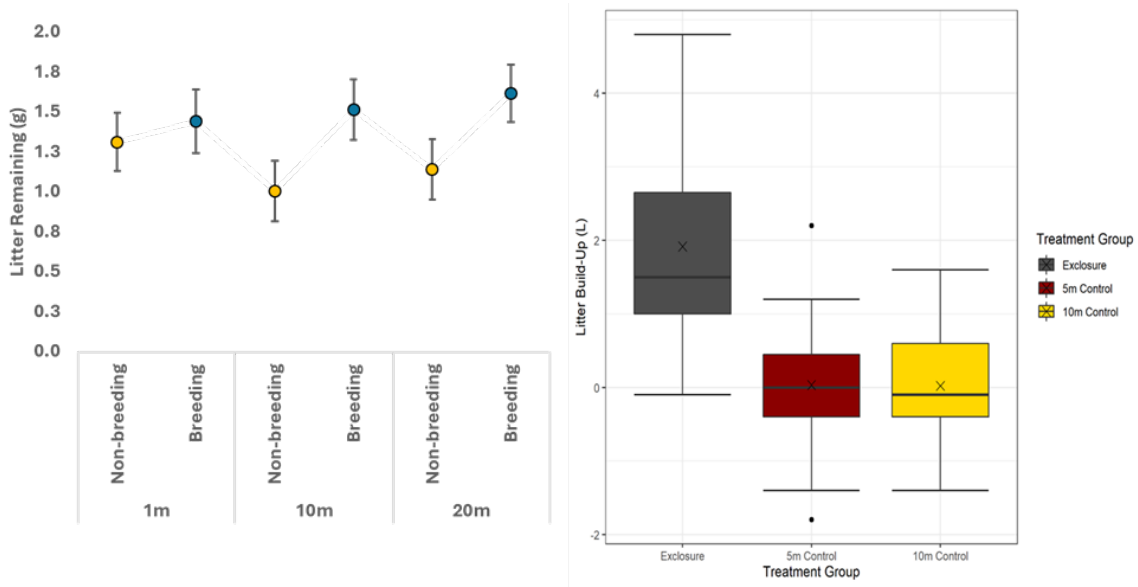
As much as it may be frustrating for some to see brush-turkeys return to the ecosystems from which they have been absent for decades, it is important to understand how they might affect the ecological processes

occurring in these areas. One role the brush-turkey might play, is that of an ecosystem engineer.

Ecosystem engineers are species that influence the availability of resources for other organisms through the creation or modification of habitat. Many other animals that dig and rake through soil act as ecosystem engineers in Australia. For example, digging and foraging by Short-beaked echidnas (*Tachyglossus aculeatus*), Burrowing bettongs (*Bettongia lesueur*), and Greater bilbies (*Macrotis lagotis*) increase moisture, nutrients, and carbon content in the soil. The ecosystem engineer with the most similar foraging style to brush-turkeys may be the Superb lyrebird (*Menura novaehollandiae*), which similarly rakes through leaf-litter. Lyrebird foraging is known to greatly reduce leaf litter build up, with the flow-on effect of reducing the spread and impact of bushfires.


Top left: Different types of litter bags used to measure decomposition rates; photo: M. Hall. Top right: Example of litter bags buried amongst litter layer near a brush-turkey mound; photo; M. Hall. Bottom: Example of 1 x 1 m brush-turkey exclusion plot; photo: C. Zirn.

We aimed to quantify the impact of brush-turkeys on leaf litter in urban areas through two studies in 2020 and 2022, focused on leaf litter decomposition and buildup respectively. For the first study, we buried mesh bags containing dried leaf litter around eighteen brush-turkey mounds in Sydney. Twelve bags with different mesh and litter combinations were placed around each mound, at varying distances, for 12 weeks to determine if distance to the mound affected the rate of decomposition. We found that proximity to the mound altered the seasonal rate of leaf litter decomposition, with leaf litter decomposing faster than the seasonal average when close to brush-turkey mounds. This suggests that brush-turkey mound building activity speeds up the rate of decomposition, and hence the cycling of nutrients back into the soil.




Left: Average leaf litter remaining in litter bags after 12 weeks of burial near brush-turkey mounds, error bars show 95% confidence intervals; credit: M. Hall. Right: Amounts of leaf litter building in brush-turkey exclusion plots compared to control plots, error bars show 1.5 x interquartile range; credit: C. Zirn.

The second experiment, conducted by Cameron Zirn for his Honours thesis, quantified the impact of excluding brush-turkeys from an area on leaf litter build up. To do this, 1 x 1m squares were closed off with chicken wire at 13 brush-turkey mounds to exclude brush-turkeys from raking and compared the amount of leaf litter that built up in these areas to control plots over a 1-month period. He found that an average of 2 L of leaf litter built up in the plots where brush-turkeys were excluded, compared to near zero in the plots brush-turkeys had access to, suggesting that brush-turkeys significantly reduce standing leaf litter. The results from these two studies conclusively show that mound building and foraging by brush-turkeys actively affect the leaf litter layer in the habitat they occupy by increasing the rate of leaf litter decomposition and slowing the rate of litter build up adjacent to their mounds. This effect is

likely to have significant long-term consequences for the leaf litter layer in the habitats they occupy, resulting in increased heterogeneity and patchiness as brush-turkeys rake litter into their mounds. Further research is needed to determine how this pattern affects factors such as soil nutrients and bush fire fuel load.

## An urban nuisance or an ecosystem engineer?

Many digging animals are absent or only occur in low numbers in urban areas. For example, Long-nosed bandicoots (*Perameles nasuta*) persist in Sydney only in patches of dense remnant vegetation and require a high degree of connectivity to disperse. Lyrebirds, despite having a similar foraging niche to brush-turkeys, are largely absent from urban areas entirely. Reintroducing ecosystem engineers to habitats modified by humans has been suggested as an important tool in ecosystem restoration and conservation.

Brush-turkeys have demonstrated a capacity to colonise human areas without human intervention, despite their odd mix of traits and high risk of predation from cats and foxes. In many cases, they may be the only large native digging animal present in urban reserves with remnant native vegetation. Thus, the species may be responsible for maintaining a significant proportion of the natural leaf litter and soil turnover that would otherwise not be able to continue in suburban areas. While brush-turkeys may be an unwelcome sight in suburban gardens, the very thing that annoys many people may also be an ecological blessing. Their continued presence and activity in these areas may be providing a vital ecosystem service to the native Australian flora that also survives and sometimes thrives in these highly modified landscapes.

## Further reading

Hall MJ, Martin JM, Burns AL, Hochuli DF (2023) Mound-building behaviour of a keystone bioturbator alters rates of leaf litter decomposition and movement in urban reserves. *Austral Ecology* 48, 1426-1439. https://doi.org/10.1111/aec.13409

Jones DN, Göth A (2008) Mound-builders. CSIRO Publishing, Collingwood, Victoria.

Göth A (2023) Amazing Annoying Birds. Natural Publishing, Sydney, NSW.

## About the authors

Dr Matthew Hall recently completed his PhD at the University of Sydney and is now an Assistant Project Officer for the NSW National Parks and Wildlife Service. Matthew's postgraduate research focused on how wildlife survives in urban environments, specifically how brush-turkey has adapted to urban landscapes. Matthew can be contacted by email (m.hall@sydney.edu.au).

Prof. Dieter Hochuli leads the Integrative Ecology group at The University of Sydney. He uses multiscale approaches to examine how nature thrives and survives in urban ecosystems, working on a diverse range of species.

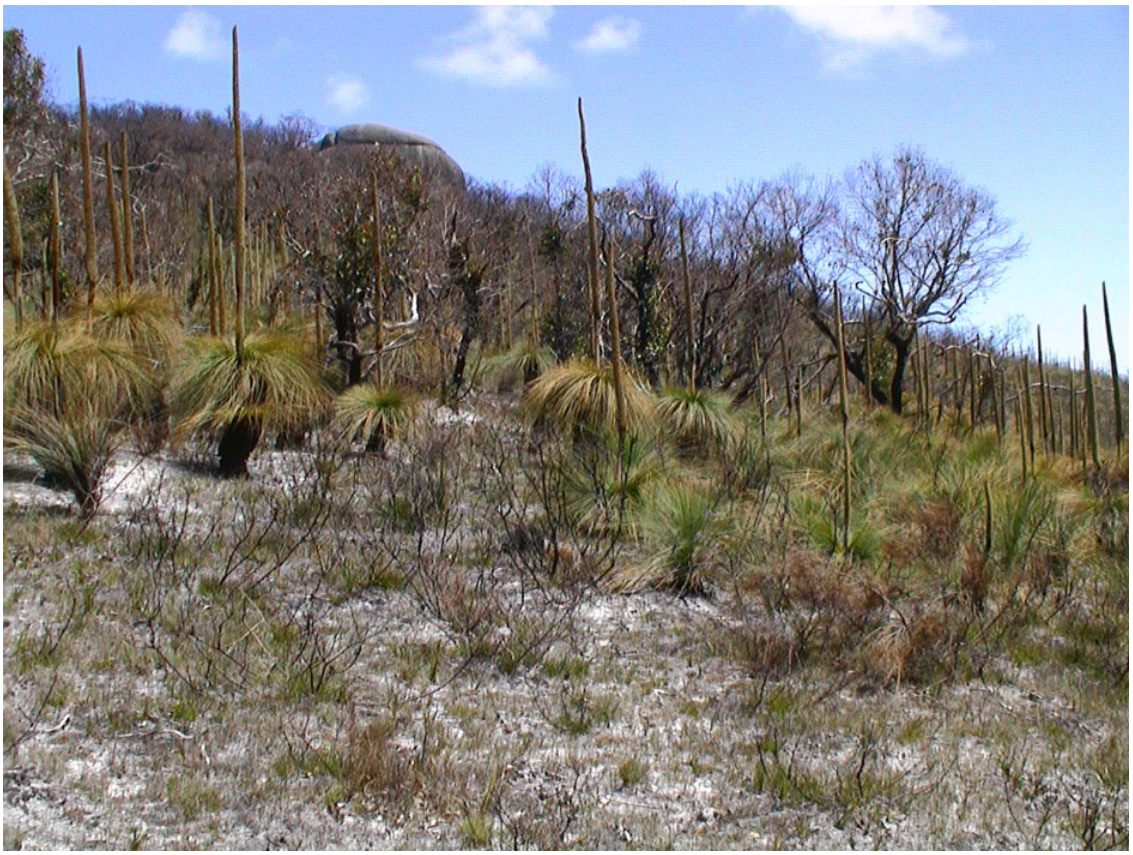
# Sniffing *Phytophthora*

David Guest[1], Ryan Tate[2], Matthew Laurence[3], and Julia Rayment[4]

[1]School of Life and Environmental Sciences, The University of Sydney, NSW; [2]Tate Animals, Port Macquarie, NSW; [3]PlantClinic, Botanic Gardens of Sydney, NSW; [4]Invasive Species Unit, NSW National Parks and Wildlife Service, NSW

## Introduction

Dieback caused by *Phytophthora cinnamomi* is a Key Threatening Process to Australia's natural ecosystems under State and Commonwealth legislation. The microscopic pathogen survives in soil and water, infects plant roots, and causes plant death, particularly in dry sclerophyll forests growing in winter dominant rainfall areas along the southeast and southwest coasts of Australia. Understorey plant communities are particularly vulnerable, and their loss causes severe habitat damage, impacts on dependent fauna and exposure of bare soil to erosion. Recent discoveries have revealed that multiple species of *Phytophthora* compound the threat to Gondwana ecosystems, including iconic species such as the Wollemi and Bunya Pines.



Dieback caused by *Phytophthora cinnamomi* at Wilson's Promontory National Park, Victoria; photo: David Guest.

## Control strategies

The most effective dieback control strategy is to prevent the introduction of the pathogen into natural ecosystems, as once introduced the pathogen spreads over vast areas through water and soil movement and is incurable. Humans are the most efficient vectors of the pathogen through the movement of contaminated soil during roadworks, mining, recreational activities, firefighting, and the nursery trade.

While protocols have been developed to limit anthropogenic spread, they depend on methods that accurately and sensitively detect and diagnose the pathogens. These methods are often impractical, expensive, and inaccurate. The standard protocol involves soil sampling and baiting, followed by morphological, serological, or molecular identification. This series of methods cost $66-$140/sample, do not include sampling costs, and take at least 1 week for processing. The sensitivity and accuracy of these techniques depends on the intensity and rigour of sampling, which for large areas rapidly becomes limiting.

In other work we have found that up to one third of the nurseries that supply seedlings for urban bushland plantings in Sydney are infected with species of *Phytophthora*. *Phytophthora* in plant nurseries causes significant direct losses from seedling deaths and indirect impacts resulting from the distribution of infected planting materials for orchard, urban and ornamental plantings, and for environmental rehabilitation projects. Disease-free nursery accreditation schemes depend on sensitive and accurate diagnosis of pathogens. For *Phytophthora* this usually requires baiting, and identification based on morphology or molecular diagnostics, adding to the cost of nursery production. Trials in California and New Zealand have shown the potential for sniffer dogs to be trained to detect volatiles indicating *Phytophthora*.

## A novel approach

In a collaboration between NSW National Parks & Wildlife Service, Tate Animals, The University of Sydney, and the Royal Botanic Gardens Sydney, funded by the NSW Government Saving our Species program, we have been investigating the potential of sniffer dogs trained to detect *Phytophthora cinnamomi* in nursery and field samples. Sniffer dogs have many uses as truffle dogs, detecting rodent pests, in biosecurity, mapping koala populations and have been used to detect *Phytophthora* in the USA and NZ, so their potential is obvious. Our primary goal is to use them to detect *Phytophthora* in nurseries that provide seedlings for bush regeneration, then to test their ability to detect *Phytophthora* in field samples.

Our research is examining if sniffer dogs detect volatiles originating from disease-stressed plants or from the pathogen. Can sniffer dogs distinguish *Phytophthora* from other pathogens? Can they distinguish *P. cinnamomi*

from other species? How cost-effective are sniffer dogs compared to conventional diagnostics? What are the most effective sampling strategies to use sniffer dogs in combination with conventional diagnostics? Can they be used as a pre-screen of nurseries in combination with conventional diagnostics to reduce sampling costs?

So far, we have trained two dogs, Echo and Alice, from 8 weeks of age using millet seed colonised with *Phytophthora cinnamomi*. Excluding training costs, we estimate a capacity of 1,000 tests each day at a cost of $1,300. We have shown that the dogs reliably distinguish seedlings infected with *P. cinnamomi* from uninoculated millet seed and from millet seed colonised with other species of *Phytophthora* or related oomycetes. They can detect dilutions of more than 1:1,000 (i.e., less than 1 g of millet seed in 1 kg potting mix), and in soil samples collected from infected bushland. Our next step is to test them in nurseries and at field sites that *P. cinnamomi* is known to be present. We will also test their ability to detect the pathogen on vehicles before and after washdowns as a way of limiting pathogen dispersal during routine management work.

Sniffer dogs not only offer an opportunity to improve the detection and management of *Phytophthora* in nurseries and reduce further impacts, but their magnetic personalities are a powerful tool for raising public awareness and education.

Sniffer dogs, Echo (left) and Alice (right) with their trainers Phil and Ryan Tate.


*About the author

David Guest is Professor of Plant Pathology and Chair of Horticulture at The University of Sydney. He studies the biology and management of diseases caused by *Phytophthora* in natural ecosystems and in horticulture. David can be contacted by email (david.guest@sydney.edu.au).

# Final Report: Testing of molecular markers for resistance to myrtle rust in Australian Myrtaceae

Peri Tobias

School of Life and Environmental Science, The University of Sydney, NSW

## Introduction

Most Australian myrtaceous species have been shown to be vulnerable to infection by the fungal pathogen *Austropuccinia psidii* (Beenken, 2017), causal agent of Myrtle Rust. Although a species may be susceptible to infection by *A. psidii*, a small proportion of resistant individuals are usually found in genetically diverse wild populations when tested in controlled inoculations (Morin *et al*., 2012). These variable responses suggest that resistant individuals, when identified, may be useful for replanting and breeding programs and to develop molecular markers for germplasm screening (Butler *et al*., 2016; Mamani *et al*., 2010).

A previous transcriptome analysis identified candidate genes that are associated with Myrtle Rust resistance within the east coast Australian littoral rainforest tree, *Syzygium luehmannii* (Tobias *et al*., 2018). While field specimens of *S. luehmannii* are generally resistant to disease from *A. psidii*, individual plants show variable responses under controlled inoculation. The candidate genes identified, both recognition receptors, seemed promising to investigate further as a molecular marker for resistance, both within species and potentially the wider Myrtaceae family. The receptor genes were homologs for a *Eucalyptus grandis* annotated nucleotide binding site leucine rich repeat-type resistance gene (NLR) and a receptor-like kinase (RLK) (Myburg *et al*., 2014). However, with no reference genome for *S. luehmannii* available, and the well-known problems in assembling immune receptor genes due to their repetitive structure (Steuernagel *et al*., 2020), it was determined to resolve the full sequences computationally before designing primers to test as molecular markers.

## Computational characterization (based on available sequence data)

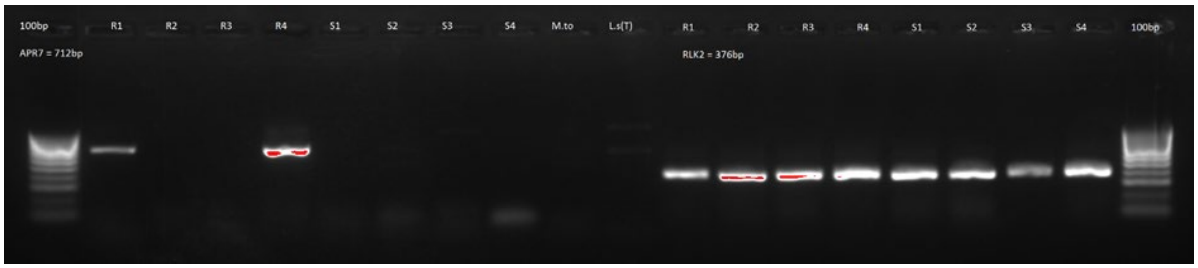
Existing RNA-seq data from inoculated plants, (National Centre for Biotechnology Information BioProject PRJNA356336) was used to make targeted new assemblies of the currently identified genes for resistance within *Syzygium luehmannii*. Custom developed python scripts were used to identify all reads that matched with the closest translated gene homolog from *E. grandis* (Eucgr.C01968 and Eucgr.K03223) and then assembled with the reads into 'genes' with trinity/2.5.1 (Haas *et al*., 2013). Outputs were multi-sequence fasta files for both putative NLR-type resistance gene (APR for *Austropuccinia psidii* resistance) and receptor-like kinases (RLK) previously noted for differential expression in resistant

plants. The primers were then tested across a range of Myrtaceae plants that were either resistant or susceptible to the rust disease.

## Primer development and testing

Based on the comprehensive assembly of the receptor genes and using the longest assembled 'transcript' in resistant plants, primers were developed and tested on cDNA. Primers were designed to span the conserved NBARC domain and the variable leucine-rich repeat (LRR) region for the receptor thereby spanning intronic regions. The expected amplicon size from DNA amplification should be larger than for the cDNA (712 bp), although without a genome for the plant, the size was not possible to predict. RNA-seq differential expression previously indicated that the putative RLK was absent in susceptible plants pre-inoculation and that the APR gene was upregulated in resistant plants at 24- and 48-hour after inoculation. The PCR amplification of cDNA, however, showed that the putative RLK gene was present in all plants, while the APR gene only amplified in two resistant plants, R1 and R4. As the original experimental plants were not able to be retained in 2017, the primers were not tested on plant DNA so further validation was not completed. Successful primers for APR were:
TGATGTAGATCATGCGAGCCAA (forward)
AACGGTTTCGGTTCCCTTCTTTTG (reverse)

Successful primers for RLK were:
GGGCAATGAGACTCCTAATACT (forward)
ACCACGCCAAAGCTATACACA (reverse)

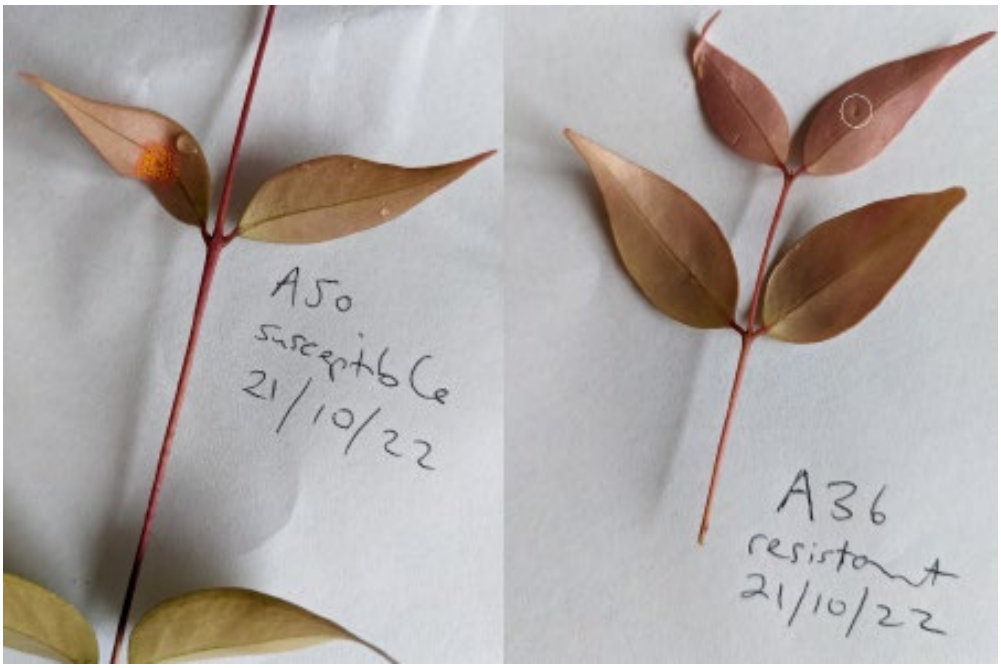



Primer testing for putative resistance (APR) and receptor-like kinase (RLK) genes using cDNA from susceptible (S) and resistant (R) plants of *Syzygium luehmannii* showing that RLK was present in all samples and APR was present in only two resistant samples (R1 and R4).

## Further screening for phenotype in response to inoculation with *Austropuccinia psidii*

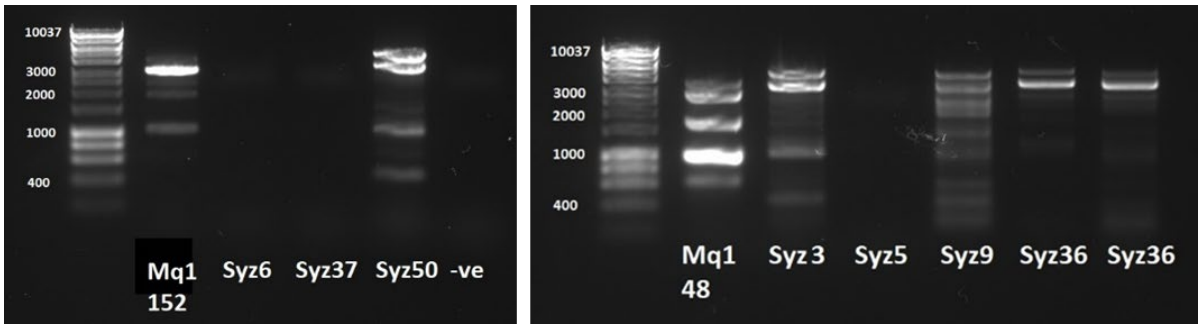
In 2018, additional *Syzygium luehmannii* plants (n = 50) were purchased, inoculated under controlled conditions, and scored for response after 10 days. From this trial, 74% of pants were susceptible and 26% were resistant. Three highly susceptible plants and four highly resistant plants were planted and grown in a garden in Manly Vale, Sydney, NSW. Cuttings were made for three further controlled inoculations. The phenotypes remained stable from every inoculation challenge and

symptoms occurred on susceptible garden planted specimens in 2022, after extended wet weather. The highly resistant plants consistently showed localised necrosis indicating rapid and successful blocking of infection.




Examples of susceptible (A50) and resistant (A36) plants of *Syzygium luehmannii* after field-exposed to Myrtle Rust. The resistant plant shows a hypersensitive response indicated by the white circle. Phenotypes were further confirmed in controlled inoculations. Scale bar approximately 1 cm.

The APR primers that were promising in cDNA resistant plants were tested against DNA from three highly susceptible (Syz 6, 37 and 50) and four highly resistant *S*. *luehmannii* plants (Syz 3, 5, 9 and 36), as well as from two plants of *Melaleuca quinquenervia* (Mq1 152 and 48) that had been screened and phenotyped. The results were inconclusive (see below) and indicate that this class of resistance genes are highly polymorphic across individuals both within the species of *S*. *luehmannii* and across another myrtaceous species.




Primer testing for putative resistance (APR) gene were tested on DNA from susceptible (left) and resistant *Syzygium luehmannii* (Syz) (right) and *Melaleuca quinquenervia* (Mq1) plants. Ladder is bp.

## Results and discussion

The results of testing molecular markers for putative resistance gene markers against *A. psidii* infection in Myrtaceae were inconclusive. The primers indicated that a specific resistance receptor (APR) was present in gene expression from two of four resistant *Syzygium luehmannii* plants and absent in all the susceptible plants, based on amplification of cDNA. While the putative gene may indeed be upregulated only in these resistant plants, it is not confirmed as a key indicator for resistance as the transcript is absent for the other two resistant plants. The putative RLK receptor amplified in all tested plant cDNA and was therefore not a useful indicator going forward. PCR amplification of the APR primers conducted on the DNA from additional phenotyped plants was also inconclusive. The non-specific binding and amplification show that the primers are complementary to several regions of DNA in many of the samples. Future work may investigate these markers in greater detail however their use as molecular markers to indicate resistance to the pathogen causing Myrtle Rust is not currently viable.

## Acknowledgements

The generous support of the Australian Flora Foundation was acknowledged at the following conferences:

2018 Queensland Molecular Biology Conference, New Zealand
2021 NSW DPI SoS Conference
2021 The Myrtle Rust Symposium, Ballina
2022 The Mycological Society

The AFF were included as industry partners in a successful ARC Linkage Grant (LP190100093) that further developed strategies to understand the impacts of Myrtle Rust. Additionally, the AFF is acknowledged as a funder for a new Myrtle Rust genome to aid plant:pathogen investigations.

Thanks to Assoc. Prof. Carsten Külheim for sharing python scripts, to Alyssa Martino for sharing *Melaleuca quinquenervia* DNA, and to Dr Jacob Downs for running some PCRs used in the analysis.

## References

Beenken L (2017) *Austropuccinia*: a new genus name for the myrtle rust *Puccinia psidii* placed within the redefined family Sphaerophragmiaceae (Pucciniales). *Phytotaxa* 297, 53-61.

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Haas BJ, Papanicolaou A, Yassour M, *et al*. (2013) *De novo* transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. *Nature Protocols* 8, 1494-512.

Mamani EMC, Bueno NW, Faria D, , *et al*. (2010) Positioning of the major locus for *Puccinia psidii* rust resistance (Ppr1) on the *Eucalyptus* reference map and its validation across unrelated pedigrees. *Tree Genetics and Genomes* 6, 953-962.

Morin L, Aveyard R, Lidbetter JR, Wilson PG (2012) Investigating the host-range of the rust fungus *Puccinia psidii sensu lato* across tribes of the family Myrtaceae present in Australia. *PloS One* 7, e35434.

Myburg AA, Grattapaglia D, Tuskan GA, , *et al*. (2014) The genome of *Eucalyptus grandis*. *Nature* 510, 356-362.

Steuernagel B, Witek K, Krattinger SG, *et al*. (2020) The NLR-annotator tool enables annotation of the intracellular immune receptor repertoire. *Plant Physiology* 183, 468-482.

Tobias PA, Guest DI, Külheim C, Park RF (2018) *De novo* transcriptome study identifies candidate genes involved in resistance to *Austropuccinia psidii* (Myrtle Rust) in *Syzygium luehmannii* (Riberry). *Phytopathology* 108, PHYTO-09-17-029.

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# Final Report: Cryo-biotechnology for conservation of endangered Sweet Myrtle (*Gossia fragrantissima* (F.Muell. ex Bently) N.Snow & Guymer)

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[1]University of Queensland, QLD; [2]Queensland Alliance for Agriculture and Food Innovation, QLD; [3]The Royal Botanic Gardens, NSW

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

## Project summary

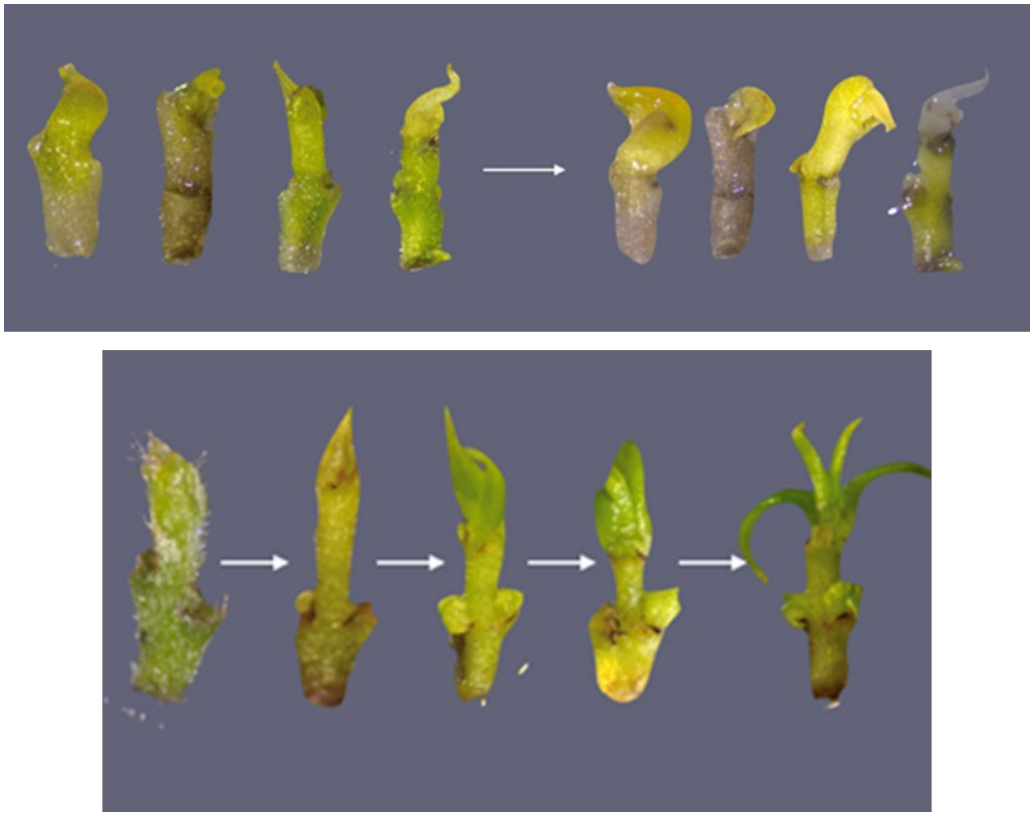
In this study, cryopreservation of *Gossia fragrantissima* shoot tips was investigated to conserve 'true-to-type plant tissue' of this endangered species. Shoot tip cryopreservation is clonal and true to the accession without any heterogeneity. The Australian Plant Bank has established shoot tip and *in vitro* multiplication protocols using sterilised cuttings from plants grown in the glasshouse. These have been successfully multiplied and supplied to The University of Queensland to develop a cryopreservation protocol.

## Excision of shoot tips

The first step in developing a protocol for cryostorage is successful excision of shoot tips in tissue culture. This is a careful process that must be done under a microscope leaving only the last few leaf primordia around the meristem. This is to allow access of cryopreservation solutions to the core meristematic cells to enable ultra-cooling without cell death. Experiments were undertaken to investigate the optimal size of excised

shoot tips needed, and a procedure was developed that enabled 100% survival and regrowth of shoots on tissue culture media post excision. The size of *G*. *fragrantissima* shoots used was 2 mm in length.

Initially, excised shoot tip extraction survival rate was 50%, however this increased to 100%. Some of the early excised shoot tips survived but failed to regrow after 4 weeks due to lack of vigour and browning. However, after improving excision techniques, all dissected shoot tips successfully grew three leaves and showed good vigour. The apical shoot tips also had axillary buds that survived after the dissection process.



Shoot tip morphology of dissected *Gossia fragrantissima* shoot tips. Top, left to right: 1 week and four weeks after dissection in early practices. Bottom, left to right: 3 days, 1 week, 2 weeks, 3 weeks, and 4 weeks after dissection and excision techniques were perfected.

## Optimisation of cryoprotectant

After development of successful shoot tip dissection, the next part of the protocol was to treat the shoot tips in a loading solution. The use of a loading solution is necessary to enhance permeation of cryoprotectant through cell membrane and to induce tolerance to dehydration by vitrification solutions (e.g., plant vitrification solution no. 2 (PVS2) and vitrification solution (VSL)). Apical shoot tips were treated with either PVS2 or VSL to determine the baseline effects of two types of cryoprotectant exposure on the survival and regrowth of the shoot tips. Both PVS2 and VSL exposure (20 min) had similar levels of negative impacts on shoot tips. Compared with the survival of dissection control

(100%), PVS2 and VSL treatments reduced survival percentages to 77% and 63%, respectively, after 2 weeks.

None of the shoot tips survived after treatment with liquid nitrogen (+LN) regardless of the cryoprotectant used. This suggested that the cryoprotection treatments were not adequate to prevent cellular death after freezing or the regrowth media was not able to support ongoing survival and regrowth of any cells that may have survived.

### Pre-treatment trials to improve shoot response to cryoprotectants

When donor plants of *G*. *fragrantissima* were subjected to cold treatment, plants showed no signs of cold damage after 2 weeks.

Shoot tips from cold-pre-treatment plants that had been through the whole cryopreservation process including LN storage (30 min) had a survival rate of 80% and 40% of shoot tips regrew. The shoot tips which were cryopreserved were slow to grow initially, however after 12 months, shoots developed into clumps and were growing as well as control plants. This provides excellent proof-of-concept that cryoprotection could be a viable method for conservation of *Gossia* species.

Left: Shoot tips of *Gossia fragrantissima* without treatment with liquid nitrogen (LN). Right: growth of shoot tips treated with LN after 12 months on regrowth media. Donor plants were pre-treated at 10°C for 2 weeks and treated with loading solution and Plant Vitrification Solution No. 2 (PVS2), with and without LN.

# 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.

## Burnley College grassland construction project – The effect of nitrogen fertility and mowing frequency on the persistence of twelve Australian perennial forbs in a planted grassland community

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

John Delpratt[1] and Ian Shears[2]

[1]The University of Melbourne, Burnley Campus, Richmond, VIC;
[2]Melbourne City Council, Melbourne, VIC

This project was funded in 1998 for $5,000 and was maintained until 2002. The AFF continues to fund research investigating the restoration of native grasslands with two new grants funded in 2023 (see "*Australian Flora Foundation Grants awarded*").

### Summary

Australian temperate native grass lands are critically endangered plant communities that typically comprise complex associations of tussock grasses interspersed with seasonally colourful forbs. Previous research has shown that the periodic reduction of the dominant grass biomass through agents such as fire and mowing has helped maintain species diversity within grassland remnants.

This project investigated the effect of two factors, soil nitrogen levels and the frequency of biomass removal, on the survival and productivity of a range of perennial native forbs in a planted grassland community.

A native grassland community comprising Kangaroo Grass (*Themeda triandra*), Common Wallaby Grass (*Austrodanthonia caespitosa*) and twelve perennial forbs was planted (using small, nursery-grown transplants) in a pre-determined pattern into a constructed, weed free, low nutrient sub-soil plot. Two frequencies of late summer biomass removal (annual; 2-yearly) and two levels of nitrogen application (none; two-monthly applications of 10 g $m^{-2}$ ammonium nitrate) were combined into four factorial treatments and maintained for four years. Biomass production of each of the planted species, and the phenology and survival of each of the forb transplants, were recorded and analysed for the duration of the experiment.

All species established well following a late autumn planting. Initially, the experimental plots were dominated by Wallaby Grass, to be replaced by Kangaroo Grass in the later years of the experiment. The survival and

growth of the various forbs varied between species, depending on their lifeform and growth habit. Only one forb species, the geophytic monocotyledon Bulbine Lily (*Bulbine bulbosa*), survived in all treatments. Several forb species survived within one or more treatments, usually on annually harvested plots with no applied nitrogen. The diversity of the forb species dropped rapidly in the later years in those plots that received nitrogen. The combination of applied nitrogen and two-yearly biomass removal quickly developed a more-or-less continuous canopy of Kangaroo Grass that excluded most other species.

Two forbs, Bulbine Lily and Native Flax (*Linum marginale*) recruited heavily from seed during the experiment, and each became an established component of the constructed grassland. No other forb recruitment was recorded. Despite the on-going presence of a colourful and diverse perennial native forb component in some burnt or mown remnants, their reliable, long-term persistence in planted grassland communities remains problematic. Future research might focus on the density of planting of grasses and forbs, the frequency and seasonal timing of biomass removal and the species composition and population size of the planted forb components.

Research relating to restoration of grasslands done by John Delpratt and others has continued since this time. Some relevant publications include:

Gibson-Roy P, Delpratt J, Moore G (2023) Coarse pine bark mulch as open surface cover fails to improve establishment of sown native grasslands. *Ecological Management and Restoration* 24, 12-19.

Gibson-Roy P, Delpratt J (2014) Meeting the seed needs for future restoration. *Australasian Plant Conservation: Journal of the Australian Network for Plant Conservation* 22, 9-10.

Gibson-Roy P, Delpratt J, Moore G (2007) Restoring the Victorian Western (Basalt) Plains grassland. 1. Laboratory trials of viability and germination, and the implications for direct seeding. *Ecological Management and Restoration* 8, 114-122.

Gibson-Roy P, Delpratt J, Moore G (2007) Restoring Western (Basalt) Plains grassland. 2. Field emergence, establishment and recruitment following direct seeding. *Ecological Management and Restoration* 8, 123-132.

Top left: The newly constructed and planted experimental area in August 1998. Top right: The 16 experimental plots were surrounded by a border planting of Weeping Grass (*Microlaena stipoides*). Bottom left: Detail of an individual plot comprising a matrix planting of Kangaroo Grass (*Themeda triandra*) and Wallaby Grass (*Austrodanthonia caespitosa*) into which were planted blocks of four seedlings of 12 grassland forbs. Bottom right: Harvested plot beginning to regrow in March 1999. The plot immediately behind was not due for harvest until the second year and had a dense stand of Wallaby Grass as the dominant species.

## An investigation of morphological variation in the salt-tolerant grass *Sporobolus virginicus* from coastal NSW and its possible application as a turf grass

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

Anthony Smith-White

School of Biological Sciences, The University of New South Wales, NSW

This project was funded in 1998 for $3,000. It is an early example of the AFF funding horticultural research to determine the potential for a native species to be used in a contemporary setting.

## Summary

The project investigated the potential of Salt Couch (*Sporobous virginicus*) for use in the turf grass industry in saline or salt affected areas. Two distinct forms of the species occur naturally in coastal areas of NSW however this study considered only the smaller form described as 'Type 1' by Smith-White (1988) and as 'var. *minor*' by others. Variation in the Type 1 form is known to be considerable with NSW populations forming part of an Australia-wide polyploidy complex (Smith-White 1979; 1981; 1988; Smith-White and Adam 1988; 1990).

The trial demonstrated a high level of genetically controlled morphological variation within the species. Such variability is potentially available for horticulturalists to draw on for specialist purposes. One accession was identified as having strong potential for cultivation as a lawn grass. The clone was collected from an estuarine sand habitat at the mouth of Coila River in southern NSW. Ecotypic adaptation in this species was well developed. Clonal spread in all chromosome races was predominately by rhizomatous growth and, while both diploid and tetraploid races were sexually fertile, the triploid race was sexually sterile. However, at times, triploids may produce seed by agamospermy (Smith-White 1988). It is likely that if a triploid accession can be used for cultivation, it would have the advantage of being genetically isolated and therefore retain its characteristics over time.

## Methods

Specific qualities are important for species to be used as a lawn grass in saline situations. These include a physiological ability to tolerate high salt concentrations and appropriate morphological traits such as stoloniferous habit, leaf size, the ability to withstand mowing, and with a turf forming character. The research funded by the AFF investigated some of the morphological traits under genetic control that are likely to be beneficial in lawn formation.

Accessions or clones of the grass (n = 36) were collected from the NSW coast between Lake Wonboyn near the Victorian border and Nambucca Heads in northern NSW. Clonal individuals were cultivated in sand in a glasshouse at the University of NSW. During the period of establishment, young root tip samples were removed for cytologically examination to determine ploidy.

Individual plants of similar size were grown in pots with coarse sand. Plants were watered twice weekly with seawater diluted 1:1 with tap water to give a 50% concentration. Plants grown for 10 weeks then measurements for the following characteristics:

- Number, height, and dry mass of tillers and stolons
- Leaf length (mean of five leaves per plant)

- Leaf width (third leaf from tip of tiller measured)
- Dry mass of roots

### Chromosome number

The cytological examination of root tip cells determined that five of the accessions were diploid, 13 were triploid, and 18 were tetraploid. The geographic distribution of these chromosome races followed that previously determined by Smith-White (1988) with the diploids coming from southern NSW, the tetraploids from northern NSW, and the triploids forming a hybrid zone between.

### Stolonifery

The clones had contrasting growth strategies with respect to tillering and stolon production. For turfing purposes, it is likely that a stoloniferous character displayed by seven clones would be advantageous.

### Leaf size

Grasses with broad leaves are considered more likely to be preferred in the turf industry because of their greater ground cover potential. Variation in leaf size was related to stoloniferous habit such that clones that were most stoloniferous generally had the broadest leaves. The triploid clone 22665 had the shortest and broadest leaves.

### Branching habit

There was an obvious correlation between stolon mass and stolon length. Four triploid clones and four tetraploid clones displayed vigorous stolon growth with a high level of branching.

Ecotypic adaptation in Salt Couch was found to be high. The research recommended that larger trials involving growth in differing salinity levels should be done to investigate the potential of naturally occurring traits for development of turf grass. Since 1998, this recommendation has been taken on by several other groups in Australia and around the world. Some recent examples of more recent research include industry funded exploratory research and in-depth molecular and genetic investigations:

Poulter RE, Bauer B (2010) Establishment and management of salt-tolerant amenity grasses to reduce urban salinity effect. Project Report. Horticulture Australia Limited, Sydney, Australia. https://era.daf.qld.gov.au/id/eprint/2177/

Rumman GA, Barrett-Lennard EG, Colmer TD (2013) Enhancing the quality of turfgrasses with saline groundwater. In: *Developments in Soil Salinity Assessment and Reclamation*, Shahid S, Abdelfattah M, Taha F (eds) Springer, Dordrecht.

Tada Y, Komatsubara S, Kurusu T (2014) Growth and physiological adaptation of whole plants and cultured cells from a halophyte turf grass under salt stress. *AoB PLANTS* 6, plu041.

Yamamoto N, Takano T, Tanaka K, *et al*. (2015) Comprehensive analysis of transcriptome response to salinity stress in the halophytic turf grass *Sporobolus virginicus*. *Frontiers in Plant Science* 6, 2015.

Endo C, Yamamoto N, Kobayashi M, *et al*. (2017) Development of simple sequence repeat markers in the halophytic turf grass *Sporobolus virginicus* and transferable genotyping across multiple grass genera/species/genotypes. *Euphytica* 213, 56.

An example of a Salt Couch (*Sporobolus virginicus*) grassland from coastal NSW (photo taken from final report).

## References

Smith-White AR (1979) Polyloidy in *Sporobolus virginicus* (L.) Kunth. *Australian Journal of Botany* 27, 429-437.

Smith-White AR (1981) Physiological differentiation in a saltmarsh grass. *Wetlands* 1, 20.

Smith-White AR (1988) *Sporobolus virginicus* (L.) Kunth in coastal Australia: the reproductive behaviour and distribution of morphological types and chromosome races. *Australian Journal of Botany* 36, 23-39.

Smith-White AR, Adam P (1988) An unusual form of the saltmarsh grass *Sporobolus virginicus* (L.) Kunth. *The Western Australian Naturalist* 17, 118-119.

Smith White AR, Adam P (1990) Chromosome number and morphotype of *Sporobolus virginicus* from coastal northwest Western Australia. *Kingia* 1, 321-325.

# Financial Report

This statement is summarised from the Foundation's audited accounts for the year ended 30 June 2023.

| **Income ($)** | **2023** | **2022** |
|---|---|---|
| Donations | 15,832 | 11,546 |
| Membership subscriptions | 1,200 | 1,530 |
| Interest | 15,874 | 21 |
| Managed fund distributions | 76,128 | 346,765 |
| Sundry income | - | 97 |
| Imputation credits | 38,308 | 43,796 |
| Increase in market value of investments | 434,118 | 43,889 |
| Bequests | 451,430 | 226 |
| Dividends CBA shares | 19,614 | 17,512 |
| Grants returned | 5,128 | - |
| **Total income** | **1,057,632** | **465,382** |
| | | |
| **Expenses** | | |
| Grants | 237,784 | 62,129 |
| Decrease in market value of investments | - | 1,647,097 |
| Audit fees | 2,690 | 2,575 |
| Administration expenses and website | 338 | 727 |
| Postage and printing | 208 | 547 |
| Young Scientist awards | 1,000 | 1,000 |
| Australian Network for Plant Conservation | - | 1,500 |
| **Total Expenses** | **242,020** | **1,715,575** |
| | | |
| **Surplus (Deficit) for the Year** | **815,612** | **(1,250,193)** |
| | | |
| **Assets** | | |
| Investments and bank accounts | 4,430,745 | 3,326,553 |
| Debtors | 36,319 | 275,801 |
| Imputation credits receivable | 32,928 | 49,364 |
| GST receivable | 19,179 | 2,652 |
| **Total assets** | **4,519,171** | **3,654,370** |
| | | |
| **Liabilities** | | |
| GST payable | 120 | 153 |
| Grant commitments | 154,898 | 105,676 |
| **Total liabilities** | **155,018** | **105,829** |
| | | |
| **Net assets** | **4,364,153** | **3,548,541** |
| | | |
| **Accumulated funds** | | |
| Accumulated funds from last year | 3,548,541 | 4,798,734 |
| Current year surplus (deficit) | 815,612 | (1,250,193) |
| **Total accumulated funds** | **4,364,153** | **3,548,541** |

# 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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- Ms Heather Miles
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- Assoc. Prof. Rachel Standish, Murdoch University, WA
- Prof. Richard Williams, University of Queensland, QLD

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