AUSTRALIAN FLORA FOUNDATION

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

Research Matters

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Inside

Welcome to new members of the AFF Council and the Scientific Committee

The AFF Council welcomes our newest member, Emeritus Professor Hans Griesser. He retired from the University of South Australia at the end of 2018 and was awarded an honorary appointment of Emeritus Professor of Surface Science. He is currently a Research Leader of the Future Industries Institute at the University of South Australia and is engaged in research projects teams at the Centre for Cancer Biology. His fields of expertise are many and varied and span chemistry and materials science, including the phytochemistry of bioactive compounds from *Eremophila*, and fundamental and applied studies of interactions between materials and biological systems, including strategies for combatting microbial infections on medical devices.

We would also like to extend a belated welcome to John Arnott who joined the Council in early 2021. John has extensive experience in the management of public gardens, landscape management principles and practices, and highly developed curatorial skills across a diverse range of plant groups and collections. He is currently Manager Horticulture at Cranbourne Gardens, Royal Botanic Gardens Victoria. Prior to this appointment John was Curator, and then Director, at Geelong Botanic Gardens. Here he was instrumental in the delivery of the 21st Century Garden project and the rejuvenation of the Geelong Botanic Gardens and its associated collections. John has also worked as Curator of Horticulture at the Melbourne Zoo. During this period, he was actively involved in the movement that took animals from cages to world class naturalistic exhibition environments, an exciting and challenging series of horticultural and landscape developments. In 2015 John was awarded the Australian Plants Award. This award is presented biennially by the Australian Native Plant Societies (Australia) to individuals who have made an outstanding contribution to the knowledge of Australian plants.

We also welcome three new members of the Scientific Committee as invaluable additions to the panel that assess small and large grant applications each year.

Dr Siegy Krauss is a Principal Research Scientist (Conservation Genetics) at the Kings Park and Botanic Gardens in Western Australia. He also holds the position of Adjunct Professor in the School of Plant Biology in the Faculty of Natural and Agricultural Sciences, University of Western Australia (since 2010). He has been a member of the Corporate Executive of the Botanic Gardens and Parks Authority (BGPA) for 18 years. In this role he provides high level strategic and policy advice on science issues impacting on the activities of the BGPA, including the management and restoration of lands under its care and the conservation of the Western Australian flora.

Dr Mark Ooi is a Senior Research Fellow in the Centre for Ecosystem Science at the University of New South Wales. He completed his PhD through The University of Wollongong in 2007 under the guidance of Prof. Rob Whelan and Dr Tony Auld. His research interests include fire and plant ecology with a focus on seed germination. His research is aimed at understanding the mechanistic responses of plant populations to different fire regimes, conservation of threatened species, and the effects of climate change in Australian plant ecosystems. Mark is a member of the NSW Bushfire Risk Management Research Hub and Threatened Species Recovery Hub and has many collaborative projects with researchers from across Australia and in Europe, China, and Brazil.

Assoc. Prof. Rachel Standish will join the Scientific Committee in 2023 once she has completed a Fulbright Future Scholarship based at the University of Wyoming, USA (August-November 2022). She is an Associate Professor in Ecology, Environmental and Conservation Sciences at the Harry Butler Institute, Murdoch University in Western Australia. Rachel is a plant community ecologist with expertise in the application of theory to restoration and conservation of native ecosystems. She co-leads the Terrestrial Ecology Research Group at Murdoch University, is a member of the Ecosystem Science Council of Australia, is a Co-Investigator of a *Drought-Net* Experimental Site and Lead-Investigator of a *Nutrient Network* Experimental Site.

Welcome everyone!

Perth’s globally unique Banksia Woodlands, a threatened ecological community in review

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Urban expansion is a risk to biodiversity at a global scale. Cities all over the world are facing the challenge of balancing urban growth and environmental conservation. A prime example of this challenge is found in Western Australia. The Perth metropolitan area is expanding at a high rate in response to population growth. This rapid growth creates a challenge for the conservation of the Banksia woodlands ecosystem that Perth is expanding into.

Banksia Woodlands are a diverse ecosystem with thousands of flora and fauna species. Many flora and fauna species occur nowhere else in the world, referred to as endemic species. Without appropriate conservation measures, many species, especially endemics, could go extinct. However, with a high number of species existing in this ecosystem, identifying what habitat and conditional needs are required to maintain all of the species can be a challenge.

Top left and right, bottom left: Remaining intact patches of Banksia Woodland around the Perth region. Images courtesy of authors. Urban expansion in Perth, Western Australia. Image from https://www.watoday.com.au/national/western-australia/halting-perth-s-urban-sprawl-is-not-as-easy-as-it-sounds-20180830-p500t1.html

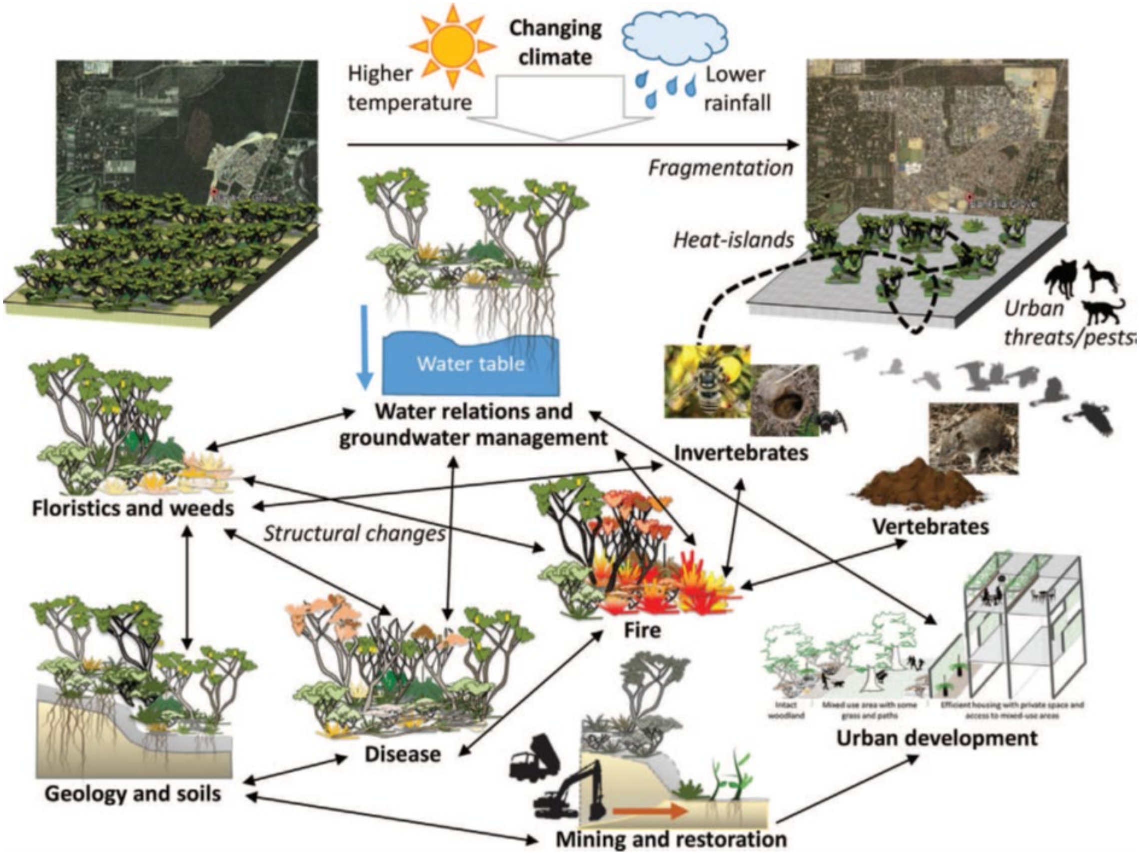
In 1989, the Royal Society of Western Australia published a review of the state of knowledge of Banksia woodland ecology and conservation in response to urban expansion that was rapidly depleting and degrading this ecosystem. Thirty years ago, when the population of Perth was approximately 0.9 million, Banksia woodlands were considered common in the metropolitan area and Swan Coastal Plain. However, by 2018, the population of Perth grew 130% to more than 2 million people. It took over 25 years (2016) for Banksia Woodlands to be listed as a Threatened Ecological Community under Australia's National environmental law, the

Environmental Protection and Biodiversity Conservation Act 1999, by which time up to 72% of the ecosystem had been lost to the development of the Perth Metropolitan region.

Andrew Burbidge said in 1989 "*If Banksia woodlands are to be used for the long-term benefit of the people of Western Australia it is clear that strategies will have to be developed and applied to prevent their total destruction or near destruction plus degradation of the remnants.*"

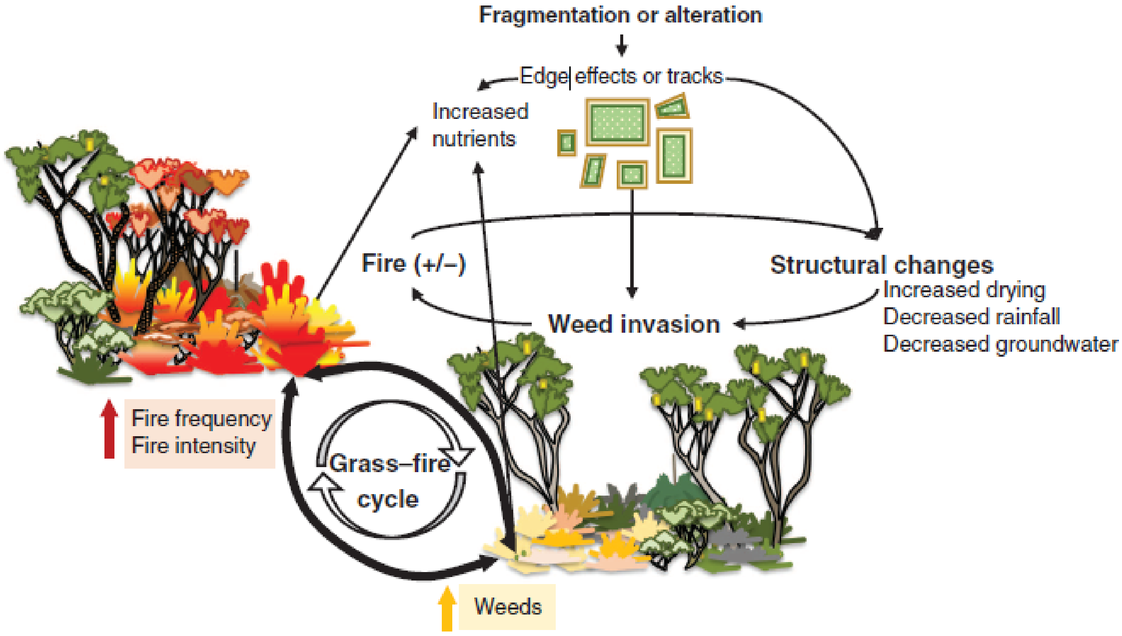
Iconic species of Banksia Woodlands in Perth, WA. Left: Inflorescence of *Banksia menziesii* (Menzies' Banksia). Photo courtesy of authors. Middle: Inflorescence of *Banksia prionotes* (Acorn Banksia). Photograph by Hesperian. Attribution-ShareAlike 3.0 Unported (CC BY-SA 3.0) https://creativecommons.org/licenses/by/3.0/. Right: Mature cone of *Banksa grandis* (Bull Banksia) Photo courtesy of John Pate.

Our recent review utilised the framework from the 1989 review (see Burbidge 1989) and presents scientific advances that have been made over the 30 years on our understanding of the composition, processes, and functions of Banksia Woodlands (Ritchie *et al*., 2021). In our review, we identify threats the ecosystem faces at present and into the future, outline what knowledge gaps exist, and identify the key research priorities that remain. Our review addresses nine main areas of scientific research within the ecosystem that were presented in the 1989 review: (1) floristics and weeds, (2) water relations and groundwater management, (3) geology and soils, (4) invertebrates, (5) vertebrates, (6) disease, (7) fire, (8) urban development, and (9) mining and restoration.



Banksia Woodlands are a complex ecosystem with high biodiversity. The review paper addresses nine main areas of scientific research focusing on this ecosystem: floristics and weeds, water relations and groundwater management, geology and soils, invertebrates, vertebrates, disease, fire, urban development, and mining and restoration. The intensity and direction of interactions between these co-occurring themes varies in both space and time and is challenged with changes in climate. Diagram from Ritchie *et al*. (2021).

Since 1989, there has been an increasing appreciation for the connectivity between these nine areas of research. As such, we added a tenth section highlighting the complexity of biotic and abiotic interactions that drive ecosystem maintenance, especially in the context of a changing climate. Ecosystem maintenance in Banksia Woodlands is the result of interactions that occur at small (meter) and large (kilometre) scales. We present a key example of alterations in fire cycles driven by exotic weedy grass species. Native species in Banksia Woodlands have adapted to specific fire-return intervals. Alterations in the return-intervals of fires pose a major risk to the maintenance of many native species.



Interactions in Banksia Woodlands demonstrating the weedy grass-fire cycle. Diagram from Ritchie *et al*. (2021).

We conclude the review with a section on future directions for research in Banksia Woodlands. A major challenge that was not discussed in the 1989 review is the impacts of a changing climate. The Swan Coastal Plain region in which Banksia Woodlands exist, is experiencing a clear warming and drying trend. This trend is further compounded by alterations in local scale land cover changes, specifically increasing heat island effect due to land clearing and infrastructure development. Similarly, the exploration and use of smart urban development that accommodates increasing population growth with reduced impacts to intact Banksia Woodlands and water resources needs to be considered. This section ends by highlighting the need for considering the human component of the ecosystem: the people of Perth. Without support from the diverse populations in the Perth Metropolitan area, the conservation of Banksia Woodlands will be a major challenge.

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Determining flower trait preferences, distribution, and seasonality of hoverflies (Diptera: Syrphidae) using observations from iNaturalist

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Introduction

Crop pollination in Australia is very much reliant on insect pollinators. Depending on the crop, insect pollination can enhance crop yield by 18-71% (Bartomeus *et al*., 2014). Most of the agricultural pollination is done by feral populations of honeybees, *Apis mellifera*, and other native insects with only a small portion provided by managed honeybees provided as a paid service (Cunningham *et al*., 2002). Such heavy reliance on honeybees is concerning given recent evidence of global declines in insect pollinators (e.g., Osterman *et al*., 2021; Zattara and Aizen, 2021).

Pest control in agriculture is most often achieved by pesticide application as it is a relatively quick and inexpensive solution. Although effective, pesticides can lead to adverse effects and potential toxicity in local wildlife and humans. Integrated Pest Management promotes the use of pest-predators and improved farming practices, which is an effective approach to controlling pest numbers while reducing usage of pesticides. Some species of native Australian hoverflies (Diptera: Syrphidae) have mobile adults that visit flowers for food and larvae that prey on aphids making them stand out as a dual service provider of both pollination and pest control (Dunn *et al*., 2020). Incorporating hoverflies into agroecosystems may provide a solution to our reliance on honeybees for pollination of crops and may contribute to safer pest management. This could be done through introduction of ‘flower strips’ which are non-crop flowering plants located adjacent to cropping fields. Perennial flower strips can increase pollinator diversity and abundance by providing additional flower resources to pollinators (Buhk *et al*., 2018).

With the ultimate aim of designing suitable flower strips, we investigated the distribution, seasonality, and floral trait preferences of hoverflies, *Melangyna viridiceps* and *Simosyrphus grandicornis*. These two species were chosen as they are commonly found throughout Australia and are known to be effective pollinators and biocontrol agents (Soleyman-Nezhadiyan and Laughlin, 1998). To do this, we used data collected through ‘citizen science’. iNaturalist is an online social platform for scientists and enthusiasts to record and share biodiversity information. The tool is designed to help with species identification, for recording locations of sightings, and cataloguing of other observations. iNaturalist also represents a potentially rich source of information that can be used for research purposes. Collecting data from a large geographical area is time consuming and expensive, citizen science provides an opportunity to increase the sampling area at low cost. However, the data collected may not be of a sufficiently high quality for research and requires careful evaluation prior to use.

Flower preferences of hoverflies

To determine flower trait preferences of hoverflies we collected data describing hoverfly sighting and information about the flowers they were visiting. Users of iNaturalist are required to upload images of the organism they have observed. Location and time data are extracted automatically from the EXIF files associate with each image. Research-grade data are observations that contain media (images), coordinates, date, and community consensus (defined as '*taxon agreed by two-thirds of identifiers*') on the identification of the species in the image.

To search for suitable images, the phrases 'Common halfband (*Melangyna viridiceps*)' and 'Yellow-shouldered stout hoverfly (*Simosyrphus grandicornis*)' were used. Only research-grade observations made within Australia that contained pictures of hoverflies on or near flowers were used in the analysis.

For hoverfly-related data, we recorded the sex of the insect displayed in each image. Sex of hoverflies was identified through dimorphism of eyes – male hoverflies have larger eyes than females with only a thin gap between the eyes, whereas females have smaller eyes with a distinct gap between them.

Sexual dimorphism of hoverflies using *Melangyna viridiceps* as an example. Left: female with smaller eyes and a noticeable gap between them (image from Jameskdouch, 2021). Right: male with larger eyes positioned close together (image from All_wall, 2022). Photos licensed under CC BY 2.0 (https://creativecommons.org/licenses/by/2.0/).

For collation of flower trait data, flower colour, shape and size were recorded. Flower size was estimated using hoverflies as a point of reference. Flowers were classified as 'small' for those smaller or equal to the size of a hoverfly, 'large' for those five times larger than the fly, and 'medium' for all flower sizes in between 'small' and 'large'. Adults of *M. viridiceps* and *S. grandicornis* have a body length of approximately 1 cm, hence a small flower was approximately 1 cm or smaller, a medium flower was 1-5 cm, and a large flower was 5 cm or larger.

Flower shape was classified using two approaches – as individual flowers and as inflorescences. This method was as it is unclear whether hoverflies perceive the shape of individual flowers, or if they perceive the overall shape of the inflorescence. Species in the Asteraceae were an exception – the shape 'ligulate' was used to describe the shape of both the individual flowers and the inflorescence. Categories of flower shapes included:

- 3D: Flowers are more densely packed, usually facing multiple directions extending from an axis; includes cluster-, cylindrical-, dome-, and sphere-shaped flowers, and those with no petals.
- Flat: The corolla of these flowers usually appears flat; flower resources are more readily available to pollinators as the foods are generally exposed; includes bowl-, circle-, ligulate-, and rotate-shaped flowers.
- Labiate: Flowers in this category are zygomorphic and have a distinct lip.

Tube: Flowers have a fused corolla or overlapping petals that form a tube; insects need to reach deep into the flower to access of nectar resources; includes bell-, funnel-, galeate-, saccate-, salverform-, and tubulate-shaped flowers.

Data quality

There were a total of 1735 observations available for *M. viridiceps* and 1047 for *S. grandicornis* in iNaturalist (up to 31/12/2021). Of these, 51% (*M. viridiceps*) and 70% (*S. grandicornis*) were of research-grade quality. For classification of hoverflies, more than 85% of observations presented hoverflies at an angle that allows identification of sex.

For the research-grade observations of hoverflies, as few as 19% and as many as 60% of images included flowers. Almost all of these observations included clearly visible flowers traits (i.e., flower colour, shape, and size). The visibility of features useful for identifying flowers to genus and species level were recorded to evaluate the potential for plant identification in insect-targeted observations. For flower-visiting hoverflies, 65% of the observations showed features of the flower, 18% showed features of the stem, and 15% showed features of leaf.

Flower shape and categories. There was a total of 16 shapes in four categories. Categories: A = 3D; B = flat; C = labiate; D = tube. Shapes: Ai = no petal + cylindrical, Aii = cluster; Aiii = dome; Aiv = sphere (images from Deborahec, 2021); Bi = bowl (image from Timojaeg, 2022); Bii = circle (image from D_kurek, 2021); Biii = ligulate; Biv = rotate; C = labiate; Di = bell (images from Romanvrbicek, 2022); Dii = funnel; Diii = Galeate (images from Chrisjonkers, 2021); Div = saccate (image from Zsims, 2021); Dv = salverform (image from Sworboys, 2021); Dvi = tubulate (image from Timmvondermehden, 2021). Photos licensed under CC BY 2.0 (https://creativecommons.org/licenses/by/2.0/).

Flower preference – size

Melangyna viridiceps frequently visited small- and medium-sized flowers (54 and 40%, respectively), as did *S. grandicornis* (58 and 32%, respectively). Less than 10% of visits were to large flowers for both species. A similar pattern was found for inflorescence size with medium-size inflorescences being preferred. Little is known about the size preference of flowers by hoverflies, but it is likely that bigger floral displays are easier to detect.

Flower preference – colour

The colours of most frequently visited flowers were white and yellow, accounting for 40% and 46% of visits by *M*. *viridiceps*, and 30% and 33% of visits by *S*. *grandicornis*, respectively. This was followed by visits to purple and pink flowers, accounting for 5-6% of visits by M. viridiceps, and 6 (pink) to 26% (purple) of visits by *S*. *grandicornis*. Flowers of other colours accounted for less than 2% of hoverfly visits. The preference for lighter coloured flowers, particularly white and yellow, has been found for other species of hoverfly. Red flowers were the least visited by both species. Instead of assuming a dislike towards red, the low frequency of interaction with red flowers is likely to be because flies are less sensitive to red wavelength ranges (Lunau and Maier, 1995).

Flower preference – shape

The most frequently visited individual flowers were ligulate- and rotate-shaped, with 46% and 36% of flowers of these two shapes visited by *M*. *viridiceps*, and 26% and 41% of flowers visited by *S*. *grandicornis*, respectively. All other flower shapes accounted for less than 10% of observations. The most frequently visited inflorescences were rotate-, ligulate-, dome-, or cluster-shaped accounting for between 12-18% of visits by *M*. *viridiceps*, and 14-26% of visits by *S*. *grandicornis*, respectively. All other inflorescence shapes accounted for less than 5% of visits.

Unlike colour, less is known about the shape preferences of hoverflies. Hoverflies have relatively short tongues and are unlikely to be able to access nectar contained at the bottom of long tubular flowers. Similarly, labiate flowers have a generally more complex structure that may render pollen and nectar resources relatively inaccessible to many hoverfly species (Babaei *et al*., 2018). The corolla of rotate-shaped flowers are relatively shallow and nectar resources are not concealed allowing insects with shorter proboscises to access them.

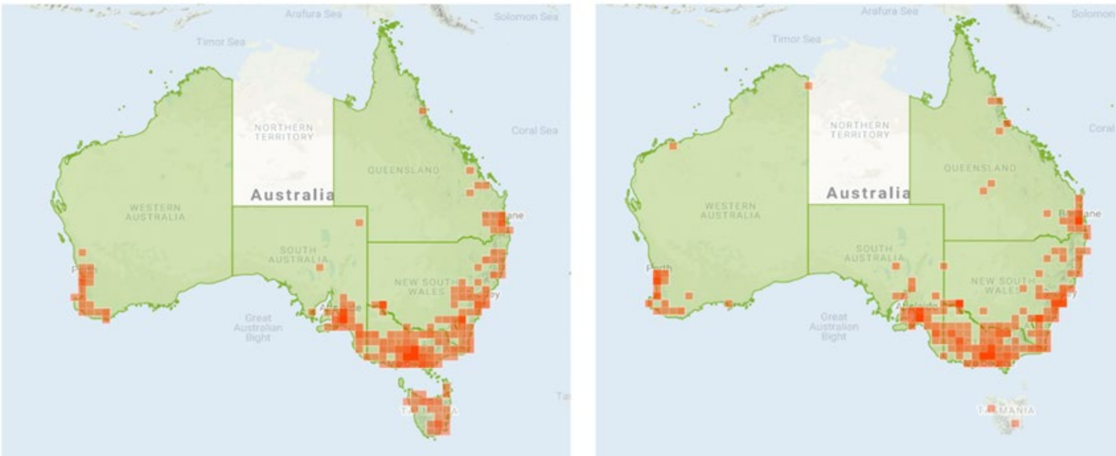
Plants with ligulate inflorescences are mostly found in the family Asteraceae. Species in this family have tubular florets that are often too deep for hoverflies with a short proboscis to access (van Rijn and Wäckers, 2016). In addition, nectar production in an inflorescence is divided across many individual florets, leading to small quantities of nectar in each floret, potentially increasing the energy required for harvesting (Carvalheiro *et al*., 2014; Babaei *et al*., 2018). Despite such shortcomings, Holloway (1976) found that over 90% of the gut contents of several species of hoverfly was pollen from Asteraceae suggesting that ligate inflorescences are an important food source for at least some species.

Nectar and pollen accessibility, as well as their nutrient composition, are important considerations when deciding what flowers to use in flower strips. Nectar accessibility determines which flowers that hoverflies can utilise, and subsequently affects their abundance and fitness (Carvalheiro *et al*., 2014; van Rijn and Wäckers, 2016).

Accessibility of nectar resources for Common halfband hoverfly, *Melangyna viridiceps*. Left: Unconcealed nectar in the flat, bowl-shaped flower of *Leptospermum* sp. (Tea Tree) (image from Reiner, 2019). Right: Concealed nectar in tubular salverform-shaped flower of *Plumbago* sp. (image from Muddles, 2020) nectar resources by. Photos licensed under CC BY 2.0 (https://creativecommons.org/licenses/by/2.0/).

Distribution and seasonality of hoverflies

The distribution of the two species of hoverflies were similar with most observations of *M*. *viridiceps* and *S*. *grandicornis* located in the south-west corner of western Australia, throughout Tasmania, and along the south-eastern coastline from southern Queensland though to eastern South Australia. No records were retrieved for the Northern Territory.



Distribution of two species of hoverflies across Australia. Left: *Melangyna viridiceps* and Right: *Simosyrphus grandicornis*. Image sourced from Atlas of Living Australia, licenced under CC BY-NC 2.0 (https://creativecommons.org/licenses/by-nc/2.0/).

In comparison, the seasonal occurrence of hoverflies was different for both species. The peak occurrence of *M. viridiceps* was in October, whereas numbers of sightings of *S. grandicornis* peaked later in November and continued to decrease during summer. Hoverflies do migrate – they are present at low latitudes throughout the year but can be found at higher latitudes during austral summer (Finch and Cook, 2020).

This study showed that citizen science, as demonstrated by observations lodged in iNaturalist, can be a useful research tool. Sourcing of information is low cost and efficient and image quality is high. In addition, for this study most observations captured sufficient plant and insect characteristics to allows different types of identification.

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*About the authors

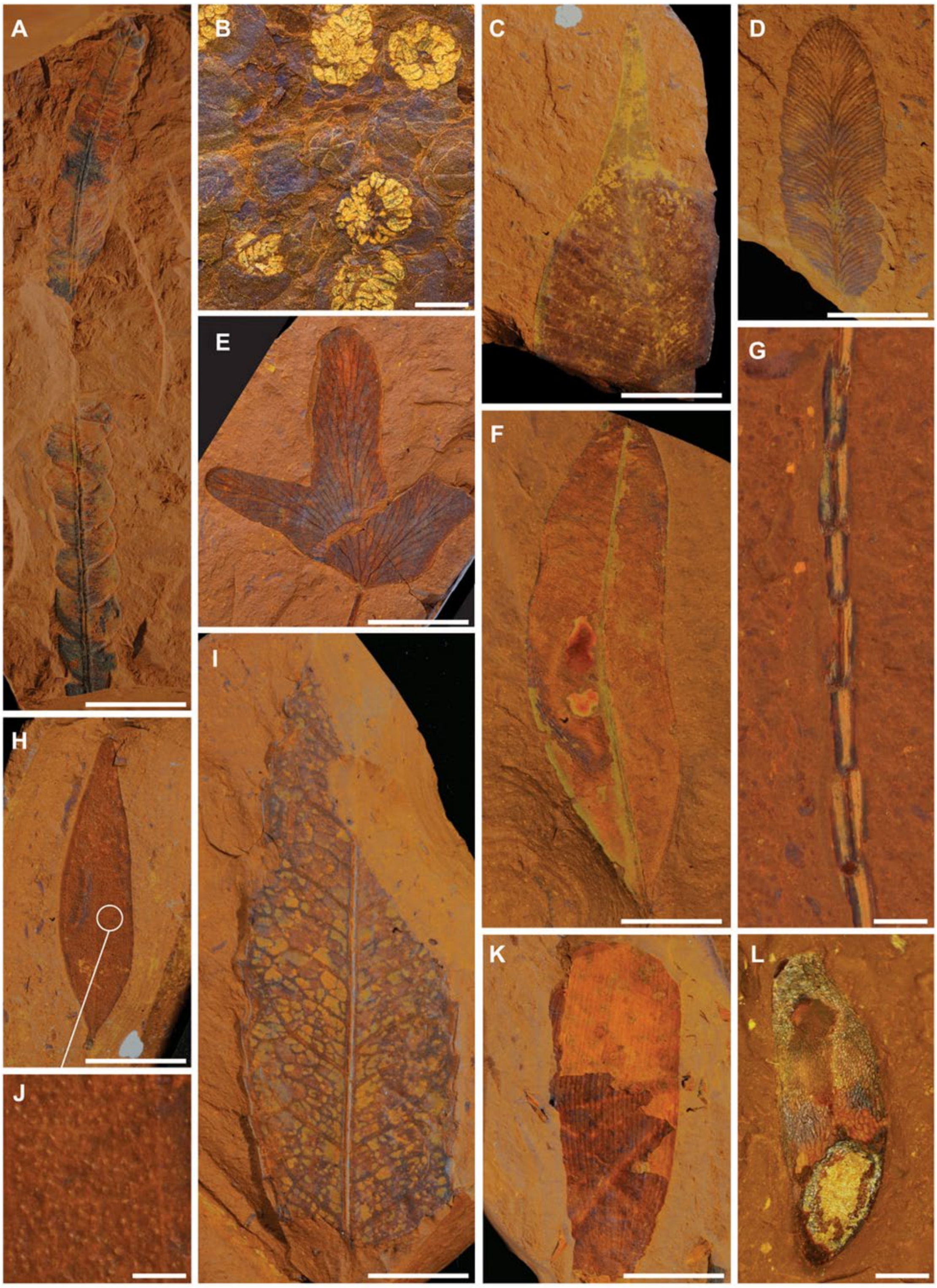
Yuen (Tiffany) Tin Sum recently completed her Bachelor of Science and Bachelor of Advanced Studies (Agriculture) at The University of Sydney. Her supervisors, Thomas White and Tanya Latty are both entomologists; Tom is an evolutionary ecologist and Tanya is interested in insect behaviour and ecology.

Australian Plants from the Eocene Era of the Miocene

Harry Loots*

It is a rare event when a whole suite of ancient Australian plants is discovered in one place. This recently occurred in ironstone slabs left lying in a field after agriculture at McGraths Flat in the Central Tablelands of New South Wales (McCurry *et al*., 2022). Fossils discovered here tell us that there was once a rainforest as well as a dryer sclerophyllous (i.e., hard leaf species) forest 25 km northwest of Gulgong. This high diversity of plants and animals dates from 37.3 ± 0.36 million years ago. Dating was achieved using Argon (40Ar/39Ar) geochronology of the nearby basalts.

This was a period before the Australian environment became progressively drier during the Miocene epoch 23.03 to 5.33 million years ago. The presence of some sclerophyllous elements and pollen that are normally associated with drier environments indicate that the rainforest was close to other habitats. These sclerophyllous elements would come to dominate the landscape as the climate became drier. The sclerophyllous elements include *Banksia* and some myrtaceous material that were likely transported from rainforest fringes or drier sites away from the main water body where fossils were formed.



Plant macrofossils. A: *Banksia* sp.; B: Isolated flowers thought to be affiliated with Malvales; C: Entire-margin leaf apex with prominent drip tip; D and E: Fern pinnules assigned to *Lygodium* sp; F: Example of myrtaceous leaf with prominent intra-marginal vein and numerous oil glands; G: Isolated twig of *Gymnostoma* sp.; H: Second example of myrtaceous leaf with prominent oil glands; I: Large toothed leaf with semi-craspedodromous venation; J: Close-up of an oil gland; K: *Agathis* sp.; L: Isolated winged samara of uncertain affinities. Scale bars are 1 mm for B, G, J, and L and 1 cm for A, C to F, H, I, and K. Image from McCurry *et al*. (2022) reproduced with permission under a Creative Commons Attribution License 4.0 (CC BY).
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There are fossils of a *Banksia* species and myrtaceous leaf with prominent intramarginal veins and numerous oil glands. Fifty different angiosperm (i.e., flowering plant) leaf taxa (i.e., species) have been found. Most of the leaf fossils are angiosperms and include Casuarinaceae (*Gymnostoma*) Proteaceae (*Banksia*), Myrtaceae (three leaf types), Lauraceae (*Cryptocarya*), Malvaceae (cf. *Argyrodendron* and *Brachychiton*), Cunoniaceae (cf. *Ceratopetalum*), and Nothofagaceae (*Nothofagus*). The most frequently found pollen is Nothofagidites representing *Nothofagus* (both *Lophozonia* and *Brassospora*). Many of Australia's present day rainforest trees are represented.

Conifers are represented by both leaves (*Agathis*) and pollen of Araucariaceae (*Araucariacites* and *Dilwynites*). There is a pollen record Podocarpidites (*Podocarpus*) and Lygistepollenites (*Dacrydium*).

Ferns are represented by pinnules (i.e., branch of a leaf), isolated sorophores (i.e., fern root growths), spores of *Lygodium*, fertile segments of *Gleichenia*, and a possible fertile filmy fern. The discovery of 14 taxa of fern spore microfossils indicates a much greater diversity, which includes Blechnaceae, Cyatheaceae, Dicksoniaceae, and Thyrsopteridaceae.

This rainforest ecosystem that once dominated the landscape has gradually transformed into the shrublands, grasslands, and deserts of today. The scarcity of such rich fossil finds has made it difficult to interpret how changes in the climate contributed to the evolution of Australia's biota and the development of modern ecosystems. The McGraths Flat site will undoubtedly contribute to our understanding of the consequences of climate change.

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*About the author

Harry Loots is a member of the Northern Beaches Group of the Australian Plants Society (APS) and the Honorary Treasurer of APS NSW, He is also a volunteer for Bushcare and Streets Alive and is an avid gardener winning awards for his native garden in 2015, 2016 and 2019.

Australian Flora Foundation Progress Report: Cryo-biotechnology for conservation of endangered Sweet Myrtle (*Gossia fragrantissima*)

Chris O'Brien and Jingyin Bao

Summary

We have been able to establish the parameters to successfully cryopreserve *G. fragrantissima* shoot tips. Work will continue in the second year of the project on improving the protocol to achieve greater than 40% regrowth after cryoprotectant treatment. Experiments to optimise rooting of excised shoot tips which have recovered after cryoprotectant will also be carried out in the second year.

In this study, cryopreservation of *Gossia fragrantissima* shoot tips was investigated to conserve 'true-to-type plant tissue'. Shoot tip cryopreservation is clonal and does not result in heterogeneity. The Australian Plant Bank has established shoot tip and in vitro multiplication protocols utilising sterilized 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 precise process that must be done under a microscope leaving only the last few leaf primordia around the meristem. This is to allow cryopreservation solutions to reach the core meristematic cells to enable ultra-cooling without cell death. Experiments were done 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. After improving excision techniques, dissected shoot tips all successfully grew three leaves and showed good vigour.

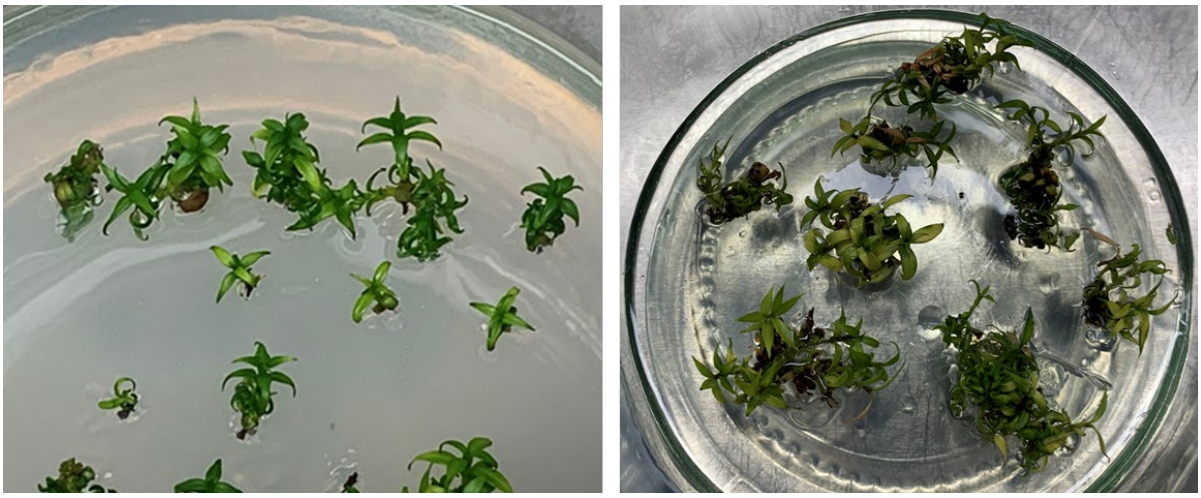
Examples of shoot tips of *Gossia fragrantissima*. Left: 'Surviving' shoot tips. Right: 'Regrowth' shoot tips. Images supplied by authors.

Optimisation of cryoprotectant

After development of a successful method for shoot tip dissection, the next part of the protocol was to treat the shoot tips in a loading solution. This is necessary to enhance permeation of cryoprotectant through cell membranes and to induce tolerance to dehydration by vitrification solutions. Compared to the survival of dissection control (100%), the two treatments used reduced survival shoot after 2 weeks. After 5 weeks, less than 25% of shoot tips had successfully regrown.

Dark treatment trials

Dark incubation of shoot tips for 1 week after being transferred onto regrowth media was trialled to evaluate if this could improve survivability. This treatment improved the survival of shoot tips following cryoprotectant treatment with a survival rate close to 100% after 1 week of treatment. However, once the shoot tips were removed from dark incubation to normal light (16 h photoperiod under LED lights), a change was observed in the viability of the shoot tips. After 2 weeks, the survival of the dark-incubated shoot tips was significantly lower than those of the shoot tips incubated in normal light.



Left: Shoot tips of *Gossia fragrantissima* 8 weeks after treatment. Donor plants were pre-treated at 10 °C for 2 weeks and shoot tips were treated with a cryoprotectant (loading solution and PVS2 minus LN). Right: The same shoot tips 8 months after treatment. Images supplied by authors.

Pre-treatment trials to improve shoot response to cryoprotectants

Our earlier work using avocado revealed that low temperature pre-treatment of donor plants in tissue culture before shoot tip extraction improved cryopreservation outcomes. We tested if the same pre-treatment was beneficial for cryopreservation of *G. fragrantissima*.

When donor plants of *G. fragrantissima* were subjected to cold treatment, shoot tips subject to cryoprotectant treatments showed no signs of cold damage after 2 weeks with survival rates of 83% and regrowth rates of

77%. Although these shoot tips were green and vigorous, they were slower growing than that of control shoot tips (i.e., cold-treated and with no cryoprotectant treatment).

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.

Stirlingia latifolia establishment
Alison Fuss and Aileen Reid, Horticultural Industries Branch, Department of Agriculture, WA
Funded in 1995 for $3,709 and in 1996 for $3,313

Propagation of *Persoonia virgarta* for the development of a new floricultural export crop
Lynda Bauer (Ketelhohn) and Margaret Johnston, School of Land and Food, The University of Queensland
Funded in 1995 for $3,839 and in 1996 for $3,672

Lomatia tasmanica and *Persoonia muelleri* propagation and commercial horticulture
Jocelyn Cambecedes and Jayne Balmer, Department of Parks, Wildlife and Heritage, Tasmania
Funded in 1995 for $5,000

The focus of these three projects was to improve understanding and propagation practices for horticulture or floriculture for species in the Family Proteaceae. All three projects showed an increase in knowledge, but commercial production of these species remains elusive. *Lomatia tasmanica* is now critically endangered so the reason for successful propagation of this species has changed substantially.

Stirlingia latifolia

At the time of this research, *Stirlingia latifolia* (Blueboy) was collected from the wild in Western Australia for the domestic market and export. The AFF-funded study investigated methods for commercial propagation and culture.

Investigation of the growth and flowering of *S. latifolia* showed the importance of fire for regeneration and flowering (Bowen and Pate, 2004) – pruning and clipping trials failed to duplicate the same regrowth. For propagation of *S. latifolia*, the best cutting material was found to be from new sprouting material following disturbance by fire. Unrooted shoots produced *in vitro* and treated with auxin showed greater establishment success that rooted shoots produced *in vitro* and treated with auxin, and were far more successful than conventional cuttings. In pot trials, *S.*

latifolia responded to the application of nitrogen, potassium and phosphorus and was speculated to mimic the growth response following fire due to a renewed supply of nutrients from ash.

Notwithstanding this improved knowledge, there has been little advancement in the cultivation of *S*. *latifolia*. In a report commissioned by the Rural Industries Research and Development Corporation (RIRDC) (Johnston, *et al*., 2000), *S*. *latifolia* was still picked from the wild at a rate of up to 3.5 million stems per annum. Stems are harvested for the display created by the fluffy husks that develop around the seeds (referred to as 'bobbles') rather than their flowers. To avoid local competition with other harvesters, stems are often picked before the fruit has developed into a full ball reducing the quality of the product. Some additional propagation research was done by the Botanic Gardens & Parks Authority, WA in the mid-2000s with investigation of large-scale production of somatic embryos for *S*. *latifolia* and other Australian native species for use in horticulture and rehabilitation of disturbed landscapes (RIRDC, 2006). Little other information is available.

Persoonia virgarta

Persoonia virgata (Small Leaved Geebung) is an understorey shrub found in coastal dry sclerophyll forest in Queensland and northern NSW. At the time of the study, flowering and vegetative stems were harvested commercially from wild populations throughout the year and sold in both the export and domestic flower markets. Stems of *P*. *virgata* were first exported in 1990, with almost one million stems harvested in 1996 shipped to the Netherlands, Germany, and Switzerland. The impetus of the AFF-funded study was that cultivation of the species was restricted because of difficulties in propagation from seed or cuttings.

According to seed germination experiments done in the AFF-funded study, removal of at least half of the endocarp is essential for seed germination. The endocarp is extremely hard and thick (1-2 mm) and is surrounded by a thick mesocarp (6-8 mm). This makes removal of the endocarp difficult, and the use of pliers and hydrochloric acid was recommended. Germination success also required microbial decontamination of the seed which was eventually achieved by soaking the endocarp and seed in bleach for 2 h before culturing aseptically. For vegetative propagation, rooting success of cuttings of *P*. *virgata* was found to be highly dependent on the age of cutting material and parent genotype. With perseverance, this study showed that the propagation of *P*. *virgata* from seed and cuttings was possible but at the time was not yet possible on a large scale.

Top left: A bunch of *Stirlingia latifolia* (Blueboy) 'flowers' which are actually seed heads. Top right: Effect of poor seed set. Bottom row: Orange, red, and yellow forms of *S*. *latifolia*, photographed at late bud stage. Images from Final Report by Reid and Fuss (2005).

Several publications came directly from the AFF-funded research (e.g., Ketelhorn *et al*., 1994; Ketelhorn and Johnston, 1995; Ketelhohn *et al*., 1996; Bauer *et al*., 1999) and led to further research for *P*. *virgata* and other species in the genus (e.g., Bauer *et al*., 2001; 2004). More recent research has found that wet/dry cycling and stratification could be used to break seed dormancy of a related species, *P*. *longifolia* (Chia *et al*., 2016). Even with an ongoing research effort (see review by Emery and Offord, 2018), the commercial cultivation of this species, and other species of *Persoonia*, still requires further research.

Lomatia tasmanica

Lomatia tasmanica (King's Holly) is a critically endangered species endemic to Tasmania. There are about 500 plants in a single remaining population growing in an area of 20-30 ha (Lynch and Balmer 2004; Australian Department of Climate Change, Energy, the Environment and Water, 2022). Surprisingly, investigation of the genetic variation of individual revealed that the entire species population of *L. tasmanica* may be one genet which, if this is true, would make it one of the largest plant clones in the world (Lynch *et al*., 1998).

Left: *Persoonia virgata* (Small Leaved Geebung). Image from PlantNET (https://plantnet.rbgsyd.nsw.gov.au/HerbLink/multimedia/68/240/Persoonia%20virgata%202.jpg). Right: *Persoonia muelleri* (Mueller's Geebung). Image from iNaturlist, supplied by cowirrie, 2015-04-03 Ben Nevis 087 (CC BY-SA 2.0) (https://creativecommons.org/licenses/by-sa/2.0/).

For the AFF-funded research, a micropropagation method was developed for *L. tasmanica* using a closely related species, *L. tinctoria*, as a model. Actively growing shoots of *L. tinctoria* were collected from the field and from potted plants, decontaminated, and introduced in tissue culture. Shoot proliferation and rooting was optimised and, using the same protocol, micropropagation of *L. tasmanica* was tested. *In vitro* root initiation on micro-cuttings of *L. tinctoria* was achieved using a small number of buds but, for a commercial application, it was recommended that the method would need to be adapted.

Persoonia muelleri

Persoonia muelleri (Mueller's Geebung) is also endemic to Tasmania. There are three recognised subspecies of *P. muelleri*, each growing in different locations. *Persoonia muelleri* ssp. *muelleri* is found in in the northeast, *P. muelleri* ssp. *densifolia* grows along the south coast and on offshore islands, and *P. muelleri* ssp. *augustifolia* grows in the west of the state. The latter species is listed as rare under the Tasmanian *Threatened Species Protection Act 1995*.

Apical and axillary buds of *P. muelleri* were used to for micropropagation but all the explants died within 6 weeks. In contrast, embryos aseptically removed from seeds were successfully established *in vitro* and plantlets derived from tissue culture were able to form roots. In the study, a small number of plants were successfully transferred to the greenhouse.


Left: *Lomatia tasmanica* (King's Holly). Image from Threatened Species Link (https://www.naturalvaluesatlas.tas.gov.au/downloadattachment?id=13067&type=image&height=600&width=800). Right: *Persoonia muelleri* ssp. *muelleri*. Image from iNaturalist, supplied by cowirrie under licence CC0 1.0 Universal (CC0 1.0) (https://creativecommons.org/publicdomain/zero/1.0/).

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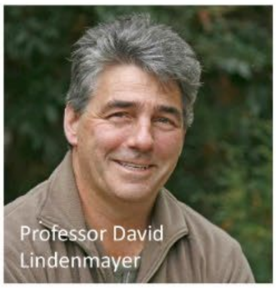

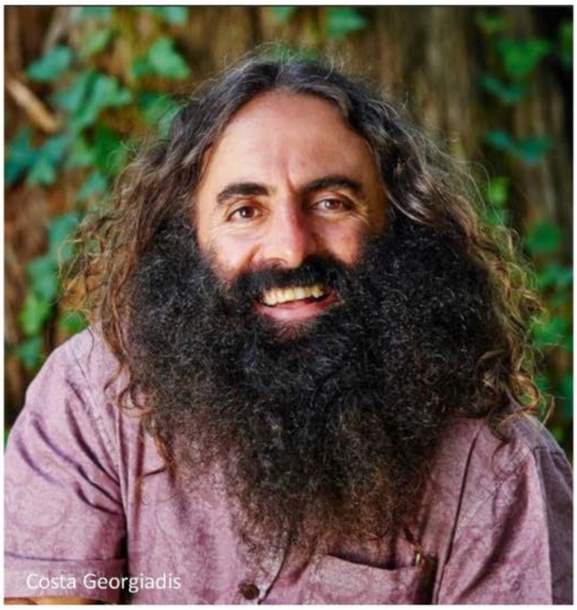

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