AUSTRALIAN FLORA FOUNDATION

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

Research Matters

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Australia’s Restionaceae, the restiads

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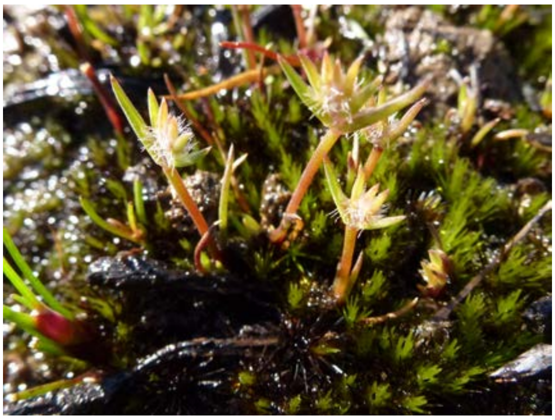
The restiads are relatives of the grasses (Poaceae) and are sometimes known as the southern rushes, since this is an almost entirely southern hemisphere family. In Australia the Restionaceae has its greatest concentration in the south of Western Australia, but there are representatives in the east and north as well. The other major concentration of the family is in South Africa, where there are even more species than in Australia.

Restiads are characteristically found on soils of low fertility and range from swamps with standing water to semi-arid, seasonally dry locations. Most are dioecious (with separate male and female plants) and the flowers are small and wind-pollinated. Some have a tufted habit and are killed by fire, regenerating by seed, while others have elongated rhizomes and resprout after fire. Often closely related species differ in this aspect, one resprouting while the other relies on seed. A good example are the three species of *Lyginea* from southern Western Australia – *L. exselsa* is killed by fire and can only repopulate an area by seed while *L. imberbis* and *L. barbarta* can resprout after disturbance such as fire (see figure below). The ability to develop different reproductive strategies has probably contributed to the species diversity.

Left: *Lyginia exselsa* forms tall dense tussocks, is killed by fire and must repopulate an area by seed; top right: *L. imberbis* also forms tight tussocks but has the ability to resprout after fire; bottom right: *L. barbarta* is a highly clonal species that resprouts after fire and rarely produces seedlings (images from T. Bell).

A few species have been used in horticulture or floristry, notably *Baloskion tetraphyllum* (Tassel Rope-rush or Feather Top) from eastern Australia and *Leptocarpus scariosus* (Velvet Rush, until recently known as *Meeboldina scariosa*; see figure below) from the south of Western Australia. Others have been used in the regeneration of mined sites. In southern Western Australia and in heaths and shrublands in parts of eastern Australia they are abundant and ecologically important constituents of the plant diversity.

Left: Habitat of *Leptocarpus scariosus* (Velvet Rush); right: the diminutive *Aphelia brizula* found only in Western Australia (images from B. Briggs).

Characteristics of restiads

For most species in the Restionaceae the leaves are reduced to sheaths with overlapping edges (unlike the fused sheaths of Cyperaceae) with scarcely any leaf blade, so most photosynthesis is in the green tissue of the stems. Where the environment varies seasonally from wet to dry, there are many different stem anatomical adaptations that tend to protect the green chlorenchyma from the stress of drying-out. In many genera, the chlorenchyma is broken into segments by cells with thickened walls, so that the stems are striated. In others there are thick-walled cells around the stomata so that evaporation is reduced.

In some species all the stems (which are often known as culms) are similar and produce flowers but in others they are of two types, with the vegetative stems shorter, more branched and more sinuous than the fertile stems.

Taxonomy of restiads

A group of tiny annual plants, as well as others in Tasmania that are cushion-forming perennials, were placed in family Centrolepidaceae, which was thought to be related to Restionaceae. Recent studies using DNA have shown, however, that this group is actually embedded in Restionaceae and should be treated as a subfamily of Restionaceae. These small *Centrolepis*, *Aphelia* (see figure above) and *Gaimardia* species resemble seedlings of other Restionaceae and it seems that one branch of the family developed the characteristic of flowering and fruiting while only at the seedling stage of development. In these plants the spikelets are also strongly modified and the flower structure is unusual. Placing the three centrolepid genera in Restionaceae, in subfamily Centrolepidoideae, has strong scientific support but has not yet been adopted everywhere. There are about 172 Australian species in the Restionaceae, 30 of them in subfamily Centrolepidoideae.

Restionaceae are divided into four subfamilies. One includes all the African genera, including the type genus *Restio*. The other three are all mainly Australian, but with about 10 species (five of them centrolepids) in New Zealand and a further 10 species reaching Chile, south-east Asia or New Guinea.

Closely related to the Restionaceae is Anarthriaceae, with three genera, *Anarthria*, *Lyginia* and *Hopkinsia*, all of which are found in southern Western Australia. These have anthers with two pollen sacs and the flowers are not in spikelets. By contrast, Restionaceae have anthers with a single pollen sac, flowers mostly in spikelets and a distinctive pollen type. Anarthriaceae is sometimes included within Restionaceae, but I recognise them as separate families because there is evidence that they have been distinct evolutionary groups for a substantial time.

Some restiad species are widespread while others are very restricted and threatened. The range of *Baloskion tetraphyllum* is from north Queensland to Tasmania and eastern South Australia, while *Leptocarpus tenax* is one of the few plant species found in both the southwest and east of the continent as well as in Tasmania. Much more limited are *Chordifex chaunocoleus* and *Loxocarya albipes*, both species are known from very narrow ranges and both have threats to their habitat. The largest Australian species is *Loxocarya gigas* with large clumps of stems reaching 1.5 to 2.5 m tall, overtopping other plants in the shrublands in Western Australia in which it occurs.

The genera of Restionaceae are distinguished by features such as the number of carpels in the ovary, fruit type and stem anatomy. Some, such as *Lepyrodia*, have 3-merous flowers with two whorls each of three tepals, 3-branched styles and the fruit is a trilocular capsule. The three stamens are in a single whorl and female flowers mostly have three small staminodes. In many genera, including *Baloskion* and *Chordifex*, the flowers are 2-merous, with four tepals, two style-branches, and the capsules are bilocular. In *Desmocladus*, *Lepidobolus* and *Catacolea*, the tepals are 3-merous but only a single carpel develops, so the style is unbranched and the fruit is a small indehiscent nut. These three closely related genera occur mostly in semi-arid habitats on sandy soils in Western Australia.

Left: Female flower of *Lepidobolus preissianus*; right: male flower of *Lepidobolus preissianus*, a species found widely in Western Australia growing on deep yellow, siliceous and clayey sands, laterite and limestone (images from B. Briggs).

On many occasions, evolution has produced similar features in different restiad genera that are not closely related. This 'parallel evolution' has made it difficult to classify species until recent studies using data from DNA sequencing clarified relationships. To reclassify species in a way that agrees with these relationships several genera have recently been united. A major change was regrouping *Leptocarpus* and *Meeboldina* so that – for example – *Meeboldina denmarkica* is now known as *Leptocarpus denmarkicus*. Similarly, *Harperia* was found not to be a natural group and its species are actually embedded within *Desmocladus*, so these have been united and *Harperia lateriflora* has become *Desmocladus lateriflorus*.

Recognising a restiad

Among the more widely known genera of Restionaceae are *Chordifex* (with 20 species), *Baloskion* (8), *Lepyrodia* (22), *Leptocarpus* (15) and *Desmocladus* (23).

Most species of *Chordifex* are from Western Australia, but four are found in the eastern states. The species found in eastern Australia differ from the others in stem anatomy, but all have a similar pattern of ridges on the seeds. Most grow in moderately well-drained sites.

Baloskion is an eastern Australian genus, with *B. tetraphyllum* being the most widespread species. Most are found in permanently damp but not inundated sites. It was surprising to find from DNA data that *Eurychorda complanata* (see figure below), the only species of its genus and found from southern Queensland to Tasmania, was only very distantly related to *Baloskion*.

Most species of *Lepyrodia* occur in seasonally or permanently wet sites, some in eastern Australia and others in Western Australia. The first species described in the related genus *Sporadanthus* was in New Zealand, but it was then realised that several Australian species, in both east and west, belong in *Sporadanthus* rather than in *Lepyrodia*. These two genera have markedly different stem anatomy.

The most widespread genus is *Apodasmia*, with four species – one of each found in Western Australia, south-east Australia (Victoria, South Australia and Tasmania), New Zealand and Chile. *Dapsilanthus*, also with four species is also widely dispersed, from northern Australia to south-east Asia (Malaysia, Cambodia, Thailand and the Chinese island of Hainan). These two genera, along with *Leptocarpus* (see figure below), all have three style-branches but only a single loculus in the ovary and the fruit is a tiny nut dispersed with the tepals attached. Wings, awns and hairs on the tepals may aid dispersal. The gynoecium in *Hypolaena* is similar but the fruits are slightly larger with the pericarp being hard and woody rather than papery.

Top left: Female flower of *Leptocarpus tenax*; top right: male flower of *Leptocarpus tenax*, a widespread species found in eastern and Western Australia; bottom left: female flower of *Leptocarpus canus*; right: female flower of *Eurychorda complanata*, the only known species in this genus, found in eastern Australia (Tasmania, Victoria, New South Wales and Queensland) (images from B. Briggs).

Arguably, the most unusual genus is *Alexgeorgea* with three species found in the south of Western Australia. The male plants have their spikelets at the tips of branches of aerial stems and, on female plants, the spikelets have only a single flower and are on very short branches from the long slender rhizomes. The base of the female flower is located belowground with only the long style with its three long stigmas projecting above the sandy soil. This is a bizarre arrangement in a group of plants that are wind-pollinated. The fruits are nuts, borne underground, and are the largest of any Restionaceae.

For further information

Hollister C, Thiele K (2014) A key to the Western Australian species in the family Restionaceae. http://florabase.dpaw.wa.gov.au/keys/

Meney KA, Pate JS (eds) (1999) Australian Rushes, Biology, Identification and Conservation of Restionaceae and allied families. University of Western Australia Press, Nedlands.

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*About the author

Dr Barbara Briggs is an Honorary Research Associate at Sydney's Royal Botanic Garden. Her career has been at the Garden, as a botanist and for many years as Senior Assistant Director, heading the science programs there. Her studies have been on the evolution and classification of several of Australia's major plant families, now using DNA data to study evolutionary relationships. She has published over 100 research papers and named 80 new plant species, as well as reclassifying others.

Using cell fusion to develop intergeneric hybrids within the *Chamelaucium* alliance

Digby Growns and Tony Scalzo
Kings Park and Botanic Garden, Western Australia

Kings Park and Botanic Garden is a world leader in the horticulture, display and scientific research into Western Australia's unique native flora through the integration of genetics, propagation science and practice, plant collecting, cultivation and landscape design.

Western Australia is internationally renowned for its spectacular flowering plants. A significant number of these species are adapted to growing in climates that have considerable periods of little or no rainfall and occur in nutritionally poor soils. Many of these plants are difficult to cultivate due to their long term adaptation to such specialised environments. Kings Park has a dedicated plant breeding program using this flora with the aim of producing highly ornamental plant varieties that can be grown with significantly less water and nutrients, and are very hardy and easy to cultivate, providing community wide environmental benefits and broad appeal for home gardens and public landscapes.

The plant families and genera selected for this breeding program include *Grevillea*, *Boronia*, the Haemodoraceae (including *Anigozanthos*, *Conostylis* and *Macropidia*), the Goodeniaceae (especially *Scaevola* and *Lechenaultia*), the *Chamelaucium* alliance (which also includes *Verticordia*, *Pileanthus*, *Darwinia*, *Actinodium* and *Homoranthus*), and flowering gums (*Corymbia* and *Eucalyptus*).

The breeding techniques used in the Kings Park program include selection of new forms and colours from natural populations, controlled pollination and biotechnology. Controlled pollination is the main method used at Kings Park for producing new cultivars, particularly in genera where fertility can be maintained in interspecific hybrids over a number of generations. Such genera include *Grevillea*, *Boronia*, *Anigozanthos* and *Corymbia*. This enables highly varied and unique cultivars to be produced through the interaction and recombination of the genomes of both parents from the second generation on.

However, in genera such as *Chamelaucium* interspecific and intergeneric hybrids have to date proved to be sterile. Kings Park has generated a number of sexually derived sterile F1 wide crosses in *Chamelaucium* such as *C. uncinatum* X *C. megalopetalum*, *C. uncinatum* X *C. lullfitzii*, *C. megalopetalum* X *C. lullfitzii* and *C. uncinatum* X *C. ciliatum*. Hybrids between *Chamelaucium* and *Verticordia* such as *C. uncinatum* X *V. grandis*, *C. uncinatum* X *V. hughanii* and *C. uncinatum* X *V. plumosa* produced through controlled pollination are also sterile.

The *Chamelaucium* alliance has its centre of diversity in Western Australia, with 260 of the 305 taxa within the alliance endemic to the state. Commonly known as waxflower, the *Chamelaucium* genus comprises 31 species. Geraldton Wax, *C. uncinatum* is the most widely known, sold internationally as a cut flower and grown widely as a garden and landscaping plant. It is a highly variable species, growing as a single trunked plant to 5 m through to compact branching forms to 20 cm, with flowering in different populations from June through to December.

Hybrids between this species and *C. megalopetalum* are popular due to their flowering display and extended flowering, and crosses between *C. uncinatum* and *C. floriferum* are renowned for their hardiness. Flower colour in waxflower is currently limited to purple (magenta), mauve and white, sometimes aging to red. Other genera within the alliance have a wider range of colours and leaf forms and offer the opportunity of greatly enhancing the attributes currently available in waxflower and sexually-derived hybrids.

Examples of species in the *Chamelaucium* alliance (image from D. Growns).

Of particular interest is capturing the red and yellow colours from *Verticordia* and the electric colours from *Pileanthus* in easy to cultivate hybrids. Sexual hybrids between *Chamelaucium* and red flowered *Verticordia* produce similar colours to *Chamelaucium*. This is because the magenta colours in *Chamelaucium* produced through the anthocyanin pathway are dominant over the red anthocyanins in *Verticordia*. Because these sexual hybrids are sterile, getting second generation hybrids that express the red colour through segregation of colour genes is not possible.

Left: An existing *Chamelaucium uncinatum* x *C. megalopetalum* 'pearl-flower' style hybrid; right: *Verticordia etheliana* var. *etheliana*, is a donor of bright red colour for cell fusion approaches to hybridisation (images from D. Growns).

To overcome the barriers to producing fertile wide crosses by sexual hybridisation somatic hybridisation through cell fusion provides scope for using species from related genera to produce novel cultivars. Somatic hybridisation involves fusion of protoplasts (plant cells with cell wall removed) from two different parents. Somatic hybrids will not grow unless the genomes are compatible, so can only be produced with related plants. Cell fusion technologies have been used in a range of breeding programs including potatoes, brassica, rice, rapeseed, tomato and citrus.

Protoplasts are derived from leaf tissue or embryogenic or cell suspension cultures treated with enzymes. They are purified and grown in osmotic media to maintain cell integrity in the absence of cell walls. The fusion process is done chemically using polyethylene glycol or through electrofusion using low voltage AC and DC currents.

Because there is no formation of gametes in cell fusion, these hybrids are polyploid, having two extra sets of chromosomes, one from each parent. Recent research suggests the polyploid state has been a major factor in plant evolution and is prevalent in flowering plant species. The production of polyploids can result in increased fertility in fused hybrids. In contrast, progeny from wide crosses produced by sexual hybridisation are usually sterile. The technique of protoplast fusion can be used to regenerate fertile tetraploid versions of these existing inter-specific and inter-generic F1 hybrids.

Fused hybrid cells are regenerated into whole plants through microcallus and macrocallus phases which subsequently have hormones applied for adventitious shoot induction or the formation of somatic embryos. Adventitious shoots are subcultured onto root initiation media and whole plants generated for deflasking, while somatic embryos germinate in a similar manner to seeds.

The research to date on cell fusion within the *Chamelaucium* alliance has shown that the development of somatic hybrids in this plant group is only possible where one parent line can be propagated through somatic embryos. Somatic embryos are a type of tissue where an embryonic structure is formed by applying various hormones. These structures are like a seed embryo in that both shoot and root meristems are present however, unlike seeds, they are clonal. That is, all somatic embryos derived from a single source will be the same, similar to cutting derived plants.

Fused hybrids between a sexually-derived *C. uncinatum* X *C. megalopetalum* and the red flowered *Verticordia etheliana* var. *etheliana* were successfully produced in 2013. These fused hybrids were germinated via somatic embryos and four of these hybrids are now being assessed for growth and flowering. The pollen from the flowers will be tested for viability and, if viable, will be used for sexual hybridisation with other fused hybrids or tetraploids with the red colour in their background, with the eventual aim of producing easy to grow, hardy red-flowered hybrids.

The application of cell fusion-based approaches offers significant scope for the introduction of novel character traits into ornamental flowering plants, and is being applied to crosses between

Chamelaucium and other related species in the genera *Verticordia* and *Pileanthus* to introduce additional bright colours and metallic floral characteristics.

In the war on weeds, farmer first-hand know-how is the secret weapon

Associate Professor Jennifer Firn*
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Every year, it is estimated that weeds (native and non-native) cost Australian farmers around $1.5 billion in control activities and lead to lost agricultural production totalling a further $2.5 billion. Many of these invasive, non-native plants contribute significantly to global environmental change and biodiversity decline. The dominance of invasive grasses can have profound impacts on agricultural communities intensifying management practices. This includes increasing landholders' efforts, increasing the costs of livestock production and, as a consequence, hardening entire landscapes ecologically, socially and economically. As more than half of Australia's land is under some kind of farmed management, this presents a major threat to the sustainability of our rural industries and health of the environment.

Scientific research on the impacts and control methods for non-native plants lag behind landholder observations and experiences. It is those who live on the land that are usually first to spot new weed incursions, and whose livelihoods depend on the decisions made locally and regionally in response to these new incursions. Given the fluctuating nature of invasive plant populations over time, robust experiments and observational research realistically necessitate long time periods to generate evidence and make useful recommendations. In the meantime, populations of invasive plant species expand and farmers, other landholders and natural resource managers have to make decisions and initiate a process of gathering knowledge through trial and error, personal observations and interactions with others.

Our research into control solutions for the non-native, invasive grass known as African Lovegrass (*Eragrostis curvula*) involved the development of a novel 'landholder first' philosophical approach for research on invasive species. African Lovegrass is unpalatable to grazing animals, if livestock do feed on it they show poor weight gain, and it is a highly competitive species with native plant species, having a faster growth rate.

The approach systematically collected observational information from landholders and learned from their experiences on the land. Experiments were then developed and/or observational surveys to evaluate these 'historically-enriched' sets of hypotheses. The first step was to elicit knowledge from landholders concerning the ecological characteristics of this grass species introduced from Africa into Australia. After compiling landholder knowledge and experiences, several common landholder derived theories emerged.

  

Left: Examples of introduced grass species *Eragrostis curvula* (African Lovegrass) (image from http://agriculture.vic.gov.au); middle: *Pennisetum clandestinum* (Kikuyu) (image from https://weeds.brisbane.qld.gov.au); right: native grass species *Themeda triandra* (Kangaroo Grass) (image from T. Bell).

We conducted a large-scale field study across 57 sites in the region of Bega Valley, New South Wales to test these hypotheses. The area is recognised as a critically endangered ecological community under the *Australian Federal Government Environmental Protection and Biodiversity Conservation (EPBC) Act* 1999.

Our research found three main benefits of incorporating local ecological knowledge into the design of research. Firstly, this approach allows for the development of a set of management recommendations supported by landholder perceptions and scientific studies. Secondly, it allows for the identification of landholder perceptions that are not supported by data and have the potential to produce negative impacts if the perceptions were to continue. Finally, it identifies landholder perceptions that may not be immediately testable, but are potentially important insights that can form a meaningful basis of long-term monitoring and management programs.

Landholder perceptions supported by scientific studies

The following observations from landholders were supported or partially supported by the research data:

- The greater the prevalence of African Lovegrass in a particular area, the less species diversity that exists
- African Lovegrass is less abundant in areas that have canopy cover, such as where paddock trees remain in these grassy woodland ecosystems
- African Lovegrass is lower in abundance when the native C_4 grass, *Themeda triandra* (Kangaroo Grass), or the exotic C_4 pasture grass *Pennisetum clandestinum* (Kikuyu), are present
- The control method of slashing increases the abundance of African Lovegrass

- African Lovegrass dominates the seed bank when present in the pasture community

These substantiated perceptions demonstrate the value of local ecological knowledge for developing research trials. Substantiated perceptions of landholders reveal several key management recommendations. The first is the importance of maintaining a healthy cover of competitive C_4 grasses. This finding suggests two possible management strategies; firstly the sowing and maintenance of Kikuyu in production pastures where the natural state of canopy trees have been largely cleared and secondly manage and maintain Kangaroo Grass as the dominant species in less intensively managed native pastures with or without remaining tree canopy.

Paddock trees have also been identified as supporting numerous production and biodiversity values, our results suggest retaining, and possibly re-establishing woodland tree cover could be an important management strategy for managing African Lovegrass.

Landholder perceptions not supported by scientific studies

Our approach is an important step in testing and refining landholders' perceptions, and potentially ceasing ineffective management. This benefit is illustrated by the three hypotheses that were not substantiated by the field survey data:

- African Lovegrass is lower in abundance on high fertility sites
- Control method of spot spraying increases the abundance of African Lovegrass
- ALG leaves are palatable at high soil nutrient levels

A regional perspective that a high impact invasive grass like African Lovegrass will not grow on high fertility sites, or that African Lovegrass leaves are palatable at high soil nutrient levels could lead to significant degradation of large expanses of habitat, as eutrophication can lead to species loss and consequently a reduction in the stability of ecosystems. The grassy woodlands are on low fertility sites, close to the ocean and the mass use of fertilisers, particularly superphosphate, has already impacted significantly on grassy woodlands elsewhere in South Eastern Australia. Testing this commonly held idea using the observational surveys has shown that African Lovegrass is high in abundance across a wide range of soil nutrient levels, and fertiliser addition may not lead to a reduction in African Lovegrass abundance.

Another common perception was that spot spraying increases the abundance of African Lovegrass. Spot spraying was found to be effective at controlling African Lovegrass, but landholder interviews provide context to interpret these results. Interviews indicate, that landholders continuing to use spot spraying were highly dedicated to this application, in some cases using GPS to track treated plants. Therefore, the success of this control strategy may be dependent on the commitment ability of landholders.

Local perceptions that are not immediately testable, but worth further consideration

A third potential benefit of consulting with landholders first is that they identified droughts and overgrazing as two key drivers of increasing invasion and dominance of African Lovegrass. Drought and intensive grazing practices can facilitate opportunities for invasive species establishment in other parts of the world and some evidence exists of the interacting effects of drought and grazing in other parts of Australia with African Lovegrass. This could not be tested in the Bega Valley in the short-term because of the need to assess conditions and growth under various climatic conditions. This perception, however, provides impetus for the establishment of long-term experimental trials.

Developing a study to test the thresholds of plant species abundance in the lead up to drought could be highly beneficial for developing a set of recommendations that will assist landholders in making the crucial decision as to when to de-stock during a dry growing season to prevent degradation.

Overall, our 'landholder first' philosophical approach demonstrates how advantageous landholders' experiences and perceptions can be for developing more informed sets of predictions on how ecosystems are impacted by invasive plants, and for launching scientific studies that are enriched with the knowledge of local history and day to day observations of change.

For further information about the study see:
http://onlinelibrary.wiley.com/doi/10.1111/1365-2664.12928/full

For information sheets on practical recommendations for controlling African Lovegrass based on the study results see:
http://www.fsccmn.com/?p=1559XXX

*About the author

Associate Professor Jennifer Firn is a restoration ecologist specialising in management of grasslands and tropical forests. Her research focuses on developing a better understanding of how the loss of native biodiversity impacts on ecosystems and subsequently finding better ways to bring it back. Jennifer is an Editor-in-Chief of the *Journal Ecology and Evolution*.

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Grant administration, or how we spend your donations

Dr Peter Goodwin
President, Australian Flora Foundation

The Australian Flora Foundation (AFF) exists to foster research into the biology and cultivation of the Australian flora. We do this by raising money as donations and bequests, and spending it on grants. The Articles of Association specify that research is scientific research, and all projects have to be assessed by our Scientific Committee. This is an outline of how the money you donate is spent.

Setting priorities

The first decision in a grant round year is to decide what areas of research we will give priority to in the coming year. Three general areas are given priority:

- Conservation of Australian plant diversity
- Cultivation of Australian plants
- Conservation of rare and endangered plants

The actual priorities are decided at the Council meeting in November/December. For some years now the conservation area has prioritised studies on the threat of climate change to our flora. Before this the role of mycorrhiza in conservation and growth of native Australian plants was a priority. The priorities have to appeal to applicants, and so have to be in areas both they and we see as important.

Funds

At the same meeting the decision has to be made as to how much the grants will be and over what period. We have very limited funds, but in general we have to increase the size of the grants each few years, just to keep up with inflation. Currently we offer to fund projects up to $10,000 per annum for 2 years, or $15,000 for 1 year. Some years we have special donations for research in a particular area. This year the Australian Native Plants Society (ANPS) Canberra is offering a donation of $10,000 for research into cool climate plants, which is being offered within the above financial guidelines; that is to say the AFF may supplement the ANPS Canberra donation by $10,000 for a 2 year project, and by up to $5,000 for a 1 year project.

Call for preliminary applications

Within a few days of the Council meeting setting the priorities and funding for the new round of grant offers, notification of a 'Call for

Applications' is sent to the following research organisations around Australia:

- 38 universities
- 15 botanic gardens with 10 or more paid staff, or less staff if the garden is actively engaged in scientific research
- 14 State departments of agriculture/environment/primary industry/natural resources
- 3 Commonwealth-wide organisations (e.g. CSIRO, ANPC)

Because only a small number of grants can be funded, we ask for preliminary applications of two pages, short list the best half dozen, and then ask the Chief Investigator (only one, to ensure there is no division of responsibility) to submit a full application. This makes best use of the researcher's, the AFF Council's and the Scientific Committee's time. The preliminary applications are due in mid-March for projects to begin in January in the following year.

Checking preliminary applications

There are a relatively small number of groups conducting scientific research on native Australian plants, and having attracted applications from them, we must ensure they have not made any elementary mistakes. Sometimes the proposed project is not on endemic native plants or the budget does not add up or the application is too long. Often the investigators do not give references to their previous work in this area, even though it is a key part of the application.

Each application is reviewed, and where there is a mistake detected the researcher is asked to correct it and resubmit within a week. Sometimes the applicant takes advantage of this chance. Sometimes they do not.

After all the proposals are in, the applications are put in random order so that each has an equal chance to be first or last during the assessment. In addition, a summary file is compiled. The summary file provides information about the total funds requested and the total funds available (usually 20% or less of the funds requested). The preliminary applications and the summary file are sent to Councillors about a month before the April/May Council meeting.

Short listing the preliminary applications

We generally get between 16 and 30 preliminary applications. The short listing (i.e. finding the research proposals to be developed into a full application) occurs at the April/May meeting of the AFF Council. Members of the Council who have a conflict of interest with a particular proposal are excluded from the meeting during discussion of that proposal at both the short listing and full application stages.

The elimination process occurs in rounds, in each the Council goes over the (surviving) applications in numerical order. In the first round Councillors are asked, for each proposal in turn, if anyone believes that application should be one of the six short listed. There is minimal discussion. If no one supports an application it is eliminated. This saves time, and usually leads to the elimination of about half the applications. If someone supports an application they may be invited to champion that application in the second round. In the second round the knives are still out. Likely to be fatal are asking for more than we are able to offer, work on non-native species, or species that are cosmopolitan (i.e. occur naturally in other countries). Other problems include the project is seen as a compilation of information, of knowledge or of (rare) plants: valuable, but not scientific research.

By the end of the second round there are usually still many more applications than the half dozen we wish to have on the short list, and most councillors would like to fund each of them, so we have now to rank them. The questions we ask are which projects will:

- Have the greatest impact?
- Help the most species?
- Offer the best value for money?

And of the research groups, which ones have:

- The strongest reputation or publication record?
- The best proven competence in the particular area of research?

Other questions we ask include:

- Are there any weaknesses in the research plan?
- Is the work a new idea?
- Can it be completed within the time frame specified?

Each of the Chief Investigators that are not short listed are advised what issues the Council had with their proposal so that they will know what to do to have a better chance next time they apply. The short listed applicants are asked to submit a full application, usually about 6 weeks later.

Sending full applications to the Scientific Committee

The full applications are sent to the Scientific Committee, a group of five highly regarded people in research on native Australian plants. Committee members are asked for comments in a range of areas and to rank each application as:

A. Fund if possible
B. Fund if have available funds
C. Not supported for funding

These findings are reported at the August Council meeting by the Chair of the Scientific Committee, currently Professor Michelle Leishman, Macquarie University.

Deciding which applications to fund

Sometimes the report of the Scientific Committee is decisive. Where the Committee members all give a project an A, it is usually funded. If two or more of the Committee give a ranking of C then it is not considered any further. However, the final decision is made by the Council – it has happened that a project given a C by the Scientific Committee has been funded. More often, some Committee members give a project an A and some give it a B – then it is 'thrashed out' in the August/September Council meeting. The issues raised at the short listing meeting are reviewed in light of information from the full application and comments from the Scientific Committee members. Sometimes the Council is so passionate about a project that the final resolution is to fund more projects than anticipated. It is satisfying to fund such good quality work but we ensure that we will receive value for money from any additional projects.

Conditions of Grant and milestone payments

Once the funding decisions have been made the successful and unsuccessful applicants are advised. As at the short listing stage, unsuccessful Chief Investigators are advised of what the problems were. All too often the Council found no major problem, but just lacked sufficient funds to support their project.

Successful applicants and their administering institution have to sign and return a document outlining the Conditions of Grant which details the requirements of the AFF and conditions for receiving funding.

There are three stages of payment:

- Initial payment: sent once the signed Conditions of Grant is received
- Progress payment: applies to 2 year grants and is sent once a suitable progress report is received, towards the end of the first year of the grant
- Final payment: sent once a suitable final report is received

Final report

The final report is what the project delivers to you, our members and donors. It is generally presented in the form of a scientific paper and may include reference to published papers which have arisen from the project. Since few people outside major research organisations have easy access to published scientific papers, the final report usually includes some or all of the information in them, but hopefully in a more readable form.

In general, the final report arrives one or more years after the final year of the grant: firstly because the grant years are usually spent acquiring the data, and the analysis of the data takes many months into the post-grant year. Secondly, most researchers aim to publish their findings in refereed scientific journals, and are encouraged to do so by the AFF, because this will provide a permanent peer-reviewed record of the research which is accessible to the scientific community. Scientific journals do not accept previously published material, so the papers must be written, submitted, accepted and published before the results can be made available to the AFF and published on our web site.

Final reports for AFF funded project can be found at http://www.aff.org.au/. Please browse these at your leisure. They are accessible not only to you and your friends, but to everyone with access to the internet.

Final project reports

The projects described below were funded by the Australian Flora Foundation (AFF). Full reports of these and other projects funded by AFF are available at: http://www.aff.org.au/

Climate change impacts on genetically differentiated *Telopea speciosissima* (NSW Waratah) coastal and upland populations

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Submitted November 2016

Background

Changes in key climatic variables such as atmospheric CO_2, air temperature and water availability are occurring at unprecedented rates and having substantial impacts on functionality, biodiversity and productivity of terrestrial ecosystems. Because forests dominate terrestrial net primary production and play a prominent role in the global carbon cycle, understanding the capacity of woody species to cope with simultaneously changing climatic variables is critical for the management of natural resources and the conservation of biodiversity.

One of the fundamental ways that plants respond to rapid climate change in the short-term is to adjust their growth and physiology, which is thought to be particularly important for woody species with long generation times. For any given species, plant populations originating from different environments usually differ in their responses to the same environmental change. Although some progress has been made on documenting intraspecific variation in woody plant response to climate change, no studies have looked into the interactive effects of concurrently changing climatic variables on their intraspecific variation in phenotypic plasticity.

Left: *Telopea speciosissima* (NSW Waratah) (image from www.anbg.gov.au); right: measuring photosynthesis and respiration of seedlings of NSW Waratah using an infra-red gas analyser (IRGA) (image from G. Huang).

Aim of the project

This project was designed to assess the impacts of key climatic variables (i.e. CO_2 concentration, temperature water availability) on growth and physiology of woody plant populations originating from contrasting environments, with a focus on the intraspecific variation in their capacity to cope with climate change.

Research method and results

The study was conducted using two populations of *Telopea speciosissima* (Family Proteaceae) originating from climatically differentiated regions. Treatment levels (CO_2 concentration, temperature water availability) were based on predicted climatic conditions occurring within this century.

In the first experiment, the main and interactive effects of elevated CO_2 concentrations (CE) and elevated temperature (TE) on growth and physiology of the Coastal (warmer, less variable temperature environment) and the Upland (cooler, more variable temperature environment) genotypes of *T. speciosissima* were assessed. Seedlings were grown under two CO_2 concentrations (400 µl -1 and 640 µl -1) and two temperature (26/16 °C and 30/20 °C for day/night) treatments.

Both genotypes responded positively to CE (35% and 29% increase in whole-plant dry mass and leaf area, respectively), but only the Coastal genotype exhibited positive growth responses to TE (47% and 85% increase in whole-plant dry mass and leaf area, respectively) when compared with the Upland genotype (no change in dry mass or leaf area). No intraspecific variation in physiological plasticity was detected under CE or TE and interactive effects of CE and TE were also largely absent. This result does not support the hypothesis that genotypes from more variable climates (e.g. Upland populations) will exhibit greater phenotypic plasticity in future climate regimes.

In the second experiment, the main and interactive effects of CE and TE on growth and physiological responses to drought of the same two genotypes of *T. speciosissima* were investigated. Using seedlings grown under the same conditions as in Experiment 1, half of the seedlings were supplied with full watering (well-watered treatment), while the other was subjected to controlled drought/recovery cycles (drought treatment).

The two genotypes showed similar declines in growth and photosynthesis under drought conditions for all CO_2 and temperature treatments and did not exhibit differences in response to drought stress. Regardless of genotype, TE negatively affected drought resistance with seedlings from all treatments becoming physiologically stressed, Similarly, CE did not influence the capacity for drought resistance or alter the sensitivity of photosynthesis to declines in soil water content. Furthermore, CE did not ameliorate the negative effects of TE on drought response.

Overall, our results suggest that woody plant populations originating from different environments may not necessarily show intraspecific variation in response to drought under current or predicted future climates. These findings also indicate that temperature is likely to be a stronger determinant than CO_2 in affecting woody plant response to drought in the context of climate change.

Conclusions

The research addressed the main and interactive effects of changes in multiple climatic variables (i.e. CO_2 concentration, temperature, water availability) on growth and physiology of *Telopea speciosissima*, with a focus on the intraspecific variation in their responses between populations originating from different environments. Significant intraspecific variation in growth plasticity when responding to a constant mild warming (TE; ambient + 3.5–4.0 °C) was found. In contrast, populations did not differ in their growth or photosynthetic responses to elevated CO_2 concentrations or to sustained drought.

Our results suggest that temperature may be more effective than CO_2 concentrations or water availability in driving intraspecific variation in woody plant populations under future climates. The expected greater phenotypic plasticity of waratah populations from more variable environments was not found. Thus, woody plant populations originating from more variable environments may not necessarily show greater phenotypic plasticity in response to climate change.

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Understanding the distribution of genetic diversity in South Australian populations of quandong (*Santalum acuminatum*) to inform genetic resource management

Dr Patricia Fuentes-Cross
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Submitted December 2016

Background

The Quandong (*Santalum acuminatum*) has a natural distribution across southern Australia. The geographical distribution encompasses a variety of habitats ranging from coastal, high altitude ranges to desert landscapes. Many dispersal vectors contribute to the mobility of Quandong throughout the landscape, such as small mammals, birds, and humans. As an important plant for humans, the Quandong has been subject to constant trading and movement across its natural range for centuries, facilitating species mobility across very large distances. The morphology of Quadong is variable. Some specimens from the Nullarbor Plain, a geologically distinctive region shared between South Australia and Western Australia, exhibit intermediate traits between *S. acuminatun* and its sister species, *S. spicatum*. This so-called Nullarbor form is thought to be a hybrid between these species and has been noted as a good rootstock in some plantations in South Australia.

Aim of the study

Because of its long history as a resource among the people of Australia and the efforts for its continued cultivation, an assessment of the genetic diversity existing in wild populations of Quandong in South Australia was warranted. In addition, it was deemed important to determine if the Nullarbor form is indeed a hybrid. The work done for the AFF formed part of a larger project exploring genetic diversity in the Quondong and the genus *Santalum*.

Left: Mature tree; right: fruit of *Santalum acuminatum* (images from P. Fuentes-Cross).

Research method and results

Wild populations in the Nullarbor region in South Australia were collected and genotyped using High Throughput Sequencing (HTS) techniques with the Ion Torrent platform (PGM and Ion Proton) to determine potential hybrids and to estimate genetic diversity. Single nucleotide polymorphisms (SNPs) were detected and used as markers to begin to develop an understanding of the genetic diversity of Quandong.

The Nullarbor form of *S. acuminatum* does not appear to be a hybrid. We determined the baseline genetic diversity to further assess extant populations as they are quite scarce, potentially as they rely on other reproductive factors such as vegetative propagation, which can have an impact on genetic diversity.

Conclusions

As a semi-cultivate, Quandong can face challenges to its long term successful cultivation due to climate change, introduced fauna and other human-induced changes to the landscape. It is important to conserve extant wild populations to ensure the future survival of the species and related species in the genus. Our results suggest that continued research of Quandong and its relatives, particularly in the dry lands of South Australia, is necessary to preserve seed banks and, along with this measure, assist in continued improvement of Quandong in cultivation.

Future plans

Conservation efforts are urgently needed to prevent the further decline of wild populations. The remaining genetic diversity held among wild and remote populations should be further investigated and maintained for an economically sustainable use of Quandong as it is possible that it will remain difficult to fully domesticate this species. Our research highlights the need for further work, including extensive taxonomic treatment of the genus. Continuation of this study will contribute to *in situ* conservation of Quandong in areas where they occur naturally. Is important to support collaborative efforts among horticulturalists, molecular botanists, and other people interested in Australian native plants and their conservation and to be mindful of the cultural relevance behind native flora. With the affordability that HTS platforms provide, genomics work to support endangered native vegetation is now even more achievable.

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Germination requirements of the lesser known Kangaroo Paw and Catspaw taxa

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Submitted July 2016

Project summary

The Kangaroo Paw, *Anigozanthos manglesii* subsp. *manglesii*, is an iconic Western Australian wildflower. However there are 20 Kangaroo Paw and Catspaw taxa in the genus *Anigozanthos* and many of them are much less well known. The primary aim of this project was to understand the germination requirements of the lesser known Kangaroo Paw and Catspaw taxa to increase awareness of these taxa and assist in their conservation and use in horticulture and land rehabilitation. Seventeen taxa were examined in this study with the more common *Anigozanthos* taxa included for comparative purposes.

Research method and results

Germination tests were done using freshly collected seed, after burial in soil and after a period of laboratory storage. Heat treatments were also done on seeds prior to imbibition. Germination of freshly collected seeds of the 16 taxa examined was negligible or low, indicating that a high proportion of seeds were dormant at maturity. Seed burial for 1 year, commencing in the autumn after collection, was most effective at alleviating dormancy and enabling germination in combination with smoke water in *A. manglesii* subsp. *manglesii*. The burial treatment also resulted in some seeds of *A.*

flavidus germinating in combination with nitrate or smoke water and light. However, for the remaining six taxa examined, the duration and timing of burial was not an effective means of alleviating seed dormancy. Likewise, germination did not exceed 15% in any of the eight taxa buried at the time of seed maturation and exhumed 3 to 4 months later, except for seed from *A. viridis* subsp. Cataby treated with smoke water. Longer periods of burial, possibly extending over at least two summers, are probably required.

Top left clockwise: *Anigozanthos bicolor* subsp. *bicolor*; top right: *A. preissii*; Bottom left: *A. humilis* subsp. *chrysanthus*; bottom right: *A. bicolor* subsp. *decrescens* (images from K. Downes).

During storage at approximately 22 °C for 3 to 3.5 years, dormancy was alleviated and smoke water stimulated higher levels of germination than water in 11 of the 17 species of *Anigozanthos* examined. In six taxa, including lesser known taxa such as *A. manglesii* var. x *angustifolius*, *A. viridis* subsp. Cataby and the conservation priority listed *A. bicolor* subsp. *exstans*, smoke-stimulated germination exceeded 49%. Heat pre-treatment at 100 °C was also effective in promoting germination in most taxa of *Anigozanthos* except *A. rufus*.

For most taxa, germination was higher following shorter (1 h) rather than longer (3 h) incubations at 100 °C. Germination of 12 of the 17 taxa examined was >30% following the 100 °C for 1 h heat treatment, and >50% in six of these taxa. These six taxa were not all the same taxa that produced the highest levels of germination following storage and smoke treatment, and included lesser known species such as *A. bicolor* subsp. *bicolor*, *A. gabrielae* and *A. preissii*.

Conclusion

There are a number of methods that can be used to maximise the germination of these intriguing taxa of *Anigozanthos* to assist in their conservation and for use in horticulture and land rehabilitation.

About us

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

Each year the AFF 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 AFF 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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