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Cover photo: Fruits of *Persoonia pinifolia* by Jill Dark

Back cover: King or Giant Fern (*Angiopteris evecta*) with a new uncurling frond, growing alongside a stream in the Daintree Rainforest, Queensland. Keith Holmes.

Australian Plants articles

Australian Plants is published quarterly to promote knowledge of Australian plants.

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Editorial

Merle Thompson

This issue of *Australian Plants* has a dual focus.

We first celebrate the achievements of two people who have been awarded our Society's principal honour – the *Australian Plants Award*.

The award is presented every two years in two categories – professional and amateur.

We include in the journal the citations for the 2015 recipients.

For the second part of the issue we feature research into specific genera and species of Australian plants.

The Society played a major role in the establishment of the Australian Flora Foundation and the member organisations, as well as individual members, play a role in its operation and contribute to its funding. The article by Dr Peter Goodwin provides those who are not fully conversant with its aims and operations with an overview together with an example of a funded project.

Three other articles are reports of projects which are being undertaken in institutions such as botanic gardens and universities.

To a non-scientific person who has a personal interest in learning about our wonderful flora, enjoying native plants in their natural environment or growing them in their garden, such studies may seem obscure, technical and a lot of work for studying just one or two species or genera. But it is the fact that such studies are undertaken that provides us with the background information to assist us to conserve some of those species which are under threat in the wild and to grow plants which we might love to have in our gardens but which defy us in cultivation.

Of course there are not enough scientists in the relevant institutions and not enough money to support research into all species which we might like to study. Thankfully many species are more plentiful and not threatened and others are conducive to propagation. It is to our benefit that interest in and the resources to study some species are available and perhaps the findings may assist with other species as well.

These articles each report on work that requires the combination of field work, laboratory study and propagation trials and living specimens. The final article, in contrast, reports on interesting work undertaken predominantly in the field and with fossil material. All are fascinating and worthwhile projects.



Australian Native Plants Society (Australia) Inc. Australian Plants Award



Every two years the ANPSA member associations are asked for nominations of persons who are considered worthy of The Australian Plants Award. The By-Laws include the following:

“The Association shall sponsor an Award known as the Australian Plants Award which shall:

- (a) be administered by the Australian Plants Award Committee comprising the President and both Vice-Presidents
- (b) be made to persons whom the Committee considers to have made an outstanding contribution to the knowledge of Australian Plants in the following categories:-
 - (i) the contribution has been made by the recipient in the course of a notable professional career in a natural science field;
 - (ii) the contribution has been made by the recipient through primarily amateur or voluntary work or interest in Australian plants.
- (c) take the form of a medallion inscribed with the recipient's name and the year of presentation together with a relevant citation.”

The 2015 recipients were John Arnott and Peter Olde. Their citations appear over-page.



John (left) and Peter (right) after the presentation of their awards.

Professional Category – Presented to John Arnott

Biennial Conference 2015, Canberra

John Arnott has made an outstanding contribution to the knowledge of Australian plants through his professional career, particularly in the horticultural aspects of zoos and botanic gardens.

John hails from Perth in Scotland and in 1966 when he was four years old, his family migrated to Australia to live in Melbourne. Since 1984 John has gained qualifications in horticulture, parks management and business. He is a member of the executive of Botanic Gardens Australia and New Zealand and life member of Friends of the Zoos and Geelong Botanic Gardens.

Over the past 30 years, he has been able to practice both his vocation and his passion in the cultivation and display of plants in the public gardens in Melbourne Zoo, Geelong & Wilson Botanic Gardens and Royal Botanic Gardens Cranbourne.

His real passion lies with the display of Australian native flora. His 20 years at the Melbourne Zoo since 1980 involved numerous projects where native plants are the feature and focus of attention. These gardens demonstrate the linkage between wildlife habitat and biodiversity conservation and therefore focus on a range of differing elements of the Australian flora.

In 2000 John joined the team at Geelong Botanic Gardens as Curator/Director to implement the 21st Century Garden project in which native plants are the primary feature. This development is the catalyst for the rejuvenation of these magnificent gardens. A significant collection of rare and threatened species from the region was also established continuing John's commitment to plant conservation.

For a short time, John worked for the City of Melbourne where he wrote an interpretation plan for the City of Melbourne Parks and Gardens, followed by a short term at Wilson Botanic Park.

In 2008 John was appointed to his dream job, Manager Horticulture at Royal Botanic Gardens Cranbourne where, with his team of





horticulturalists, he developed stage two of the Australian Garden to focus on the eastern seaboard of the continent with its rivers, forest, coast vegetation and urban landscapes. The basic premise is to help people to understand, to value and in turn to protect our wonderful native flora as well as the landscapes and ecosystems from which they occur.

In all of his work he has applied this philosophy to demonstrate and promote that ... *"Australian native flora is not an alternative, it is something to garden with - to be inspired by. If I can contribute in any meaningful way to this agenda, then I am indeed a fortunate gardener!"*

This statement alone makes John a worthy recipient of the Australian Plants Award.

ANPSA President Lawrie Smith AM

Amateur Category – Presented to Peter Olde

Biennial Conference 2015, Canberra

Peter Olde has made an outstanding contribution to the knowledge of Australian plants, in a voluntary capacity during his membership of the Australian Plants Society of NSW for 38 years. He is an Honorary Life Member of the Society.

Peter graduated from the University of Sydney in 1967, followed by two years in the Australian Army Intelligence Corps and a further two as language teacher at Sydney Boys' High. Then followed 31 years as a self-employed company director. Peter has been an Honorary Research Director of the NSW Herbarium since 1993.

He joined the APS Sutherland group in 1977 quickly becoming President then serving in that capacity until 1982. He became leader of the Grevillea Study Group in 1980, a position he still holds. Peter has held various executive positions in APS NSW and was elected President 1988 – 1990. He was appointed an APS NSW Life Member in 1998.

In 2003 he established 'Silky Oaks' at Oakdale a private botanical garden that he planned to display native plants to their best potential. This unique garden has since become a regular participant in the Open Gardens Australia program.

As leader of the Grevillea Study Group, Peter ensures that three newsletters are distributed annually and he also completed the three



Peter Olde on a grevillea study group crawl photographing a form of *G. alpina*.

volume *Grevillea Book*. He has seen and photographed over 90% of the genus in person. His general interest and knowledge of the genus has led to the discovery and naming of many new species. Most of his taxonomic work is concentrated on the *Grevillea* of New South Wales and Western Australia. He is currently researching the taxonomy of some of the more complex *Grevillea* species.

Peter's all-consuming interest in the Proteaceae family, particularly the *Grevillea* genus, is demonstrated by reference to the numerous scientific & horticultural publications for which he has been responsible. These scientific publications cover most aspects of the Genus extending from taxonomic studies, Western Australian species, Rainforest species, Grevillea at Burrendong, Waratah, Grevillea nectar sugars, Pests & Diseases, and finally to a short history of cultivars and hybrids.

Truly a man with an unparalleled passion for the Grevillea genus! His consistent and continuing dedication to the knowledge and promotion of Australian plants makes Peter a very worthy recipient of the Australian Plants Award.

ANPSA President Lawrie Smith AM



The Australian Flora Foundation Fostering research into the biology and cultivation of Australian plants

Dr Peter Goodwin President of the Australian Flora Foundation.

Ten Facts about the Australian Flora Foundation

1. We only fund research on the biology and cultivation of Australian Plants.
2. We only fund research in Australia.
3. The Australian Flora Foundation Inc was established in 1981. It arose mainly from the desire of members of the Australian Plants Society and plant scientists to see more research carried out on Australian flora.
4. It is funded by membership fees, donations and bequests. 100% of donations and bequests goes to research.
5. The first grants were made in 1986 and grants totalling over \$500,000 have been awarded since 2000.
6. The 113th grant was made in 2016.
7. Grants have led to the establishment of whole industries, most notably the riceflower export industry.
8. Many young scientists have gone from an initial grant from the Foundation to spending their research career working on Australian plants.
9. The Foundation is run by an elected Council, whose members are often highly qualified individuals who give their time freely.
10. You can find out all the grants awarded, all the results, details of the Council, and much else besides at the Foundation's website: www.aff.org.au/

How does the AFF Council choose projects to fund?

1. At the November meeting each year the Council decides what will be the special interest categories in the next year's grants. For example, for grants starting in 2015 these were conservation of Australian plants particularly where there are threats from climate change, the cultivation of Australian plants, and rare and endangered plants.
2. A Call for Applications is then sent out to all Australian Universities, Botanic Gardens, State Departments of Agriculture/ Environment/ Conservation and the CSIRO. People are asked to put in a brief outline

(a preliminary application) of what research they propose to do, and how it would forward the interests of the Foundation.

3. The Council usually receives 25-30 preliminary applications, but can only afford to fund 3 to 4. At an April meeting the best 6 to 8 applications are chosen and these applicants are asked to put in a full application.

4. The full applications are then sent to the Scientific Committee, an independent group of eminent scientists. Any application about which the Scientific Committee has reservations is removed.

5. The final decisions are made at a Council meeting in August and the successful applicants advised that they will have funding beginning in December. Sometimes APS groups give donations on the condition that the work is on particular categories of plants, and for the researchers to give them a talk about the work. These conditions are added to the usual conditions of grant to which the researchers have to agree before the funding is sent.

To give you an idea of the type of research report the Foundation receives, the following is an abstract of a final report received after a grant of \$8,800 to Dr Rozefelds in 2002.

Australian Flora Foundation Report on the AFF Grant 001-09 – The Horticultural Potential of an endangered species, *Philotheca freyciana* (Freycinet Wax Flower), from Eastern Tasmania

Ms Natalie Papworth & Dr Alan MacFadyen, Royal Tasmanian Botanical Gardens, Hobart; Mr Alan Gray, Tasmanian Herbarium; Dr Anthony Koutoulis and Ms Aina Price, School of Plant Sciences, University of Tasmania; Dr Andrew Rozefelds, Tasmanian Museum and Art Gallery.

Abstract ¹

Surveys undertaken on the Freycinet Peninsula found over 100 plants of the critically endangered *Philotheca freyciana* (Freycinet Wax Flower). All plants are restricted to The Hazards and a single plant is also known from nearby Cape Tourville. *Philotheca freyciana* is restricted to skeletal soils derived from granitic rocks and it often occurs in runnels and in vertical crack lines in the granitic terrain. This plant is likely to be slow-growing, although both young and mature plants were located, which indicates recruitment is occurring in the wild.

¹ The reports of many projects are long and technical. This project has been chosen as an example because the report is both an appropriate length for inclusion in this article and its style of writing is more applicable to a general readership. It also brings together environmental and conservation issues with rare and endangered plants and cultivation techniques.





As the species is critically endangered a number of propagation techniques were attempted, including seed, tissue culturing and standard vegetation propagation, to establish an ex situ population. Standard nursery vegetative propagation methods were found to be the most effective and, as a result, the Royal Tasmanian Botanical Gardens (RTBG) now holds 70 cutting-produced plants in pots from various genotypes. The project has successfully established an ex situ collection of this critically endangered species at the RTBG.

Tissue culture techniques have successfully resulted in shoot proliferation, but we have been unable to induce root initiation in explants to date. If we can successfully overcome this challenge it will become possible to explore the horticultural potential of this attractive species.

Background

Philotheca freyciana (Freycinet Wax Flower) was described in 2001. This species was at that time restricted to three known plants in the wild, all localised to The Hazards and Cape Tourville on the Freycinet Peninsula in eastern Tasmania. Because of its rarity and restricted distribution it was listed as critically endangered under the Environment Protection and Biodiversity Conservation Act 1999.

This study supported by the Australian Flora Foundation aimed to:

- (a) determine the distribution of the species by undertaking surveys in the Freycinet Peninsula and other areas of similar habitat,
- (b) commence life history studies to better understand the ecology of the species, and
- (c) attempt to propagate the species through a variety of propagation techniques.

a. Survey

To extend knowledge of the ecology of this species a systematic search of the Freycinet Peninsula was carried out in 2002 which located over 100 plants in populations extending from the summits of various peaks in The Hazards to near sea level. All plants occurred on granite outcrops, often precariously over the edge of cliffs in drip lines and vertical cracks (Fig. 1). The boulder-strewn terrain makes some areas of the Freycinet Peninsula difficult to access. Searches on granitic rocks on nearby Schouten and Maria Islands failed to find any plants of *Philotheca freyciana*.

From these various surveys undertaken by staff from the Tasmanian Herbarium, Department of Primary Industries, Water and Environment

and the Royal Tasmanian Botanical Gardens it was concluded that the species is restricted to The Hazards on Freycinet Peninsula, with plants occurring on Mt Mayson, Mt Amos, Mt Dove and Mt Parsons with a single plant occurring at Cape Tourville. The results of the surveys would support the species retaining its status as being critically endangered.



Fig. 1. Field surveys involved clambering over the granitic rocks of Freycinet Peninsula looking for *Philotheca freyciana*. The plants often occurred in cracks and along runnels in the granitic rocks. This view shows the picturesque Wine Glass Bay in the background.





b. Life History Studies

From the largest population (of about 50 plants) on Mt Mayson, 35 were selected to begin a study of growth and development. All plants were measured for height, width of plant, stem diameter at ground level (Fig. 2) and observations were made as to whether the plants were in bud, flowering, or post anthesis.



Fig. 2. Measuring stem diameter of *Philotheca freyciana* plants at Mount Mayson.

The plants are generally spindly, consisting of a main stem and are often sparingly branched. The population included young plants, 4 cm in height, through to large, well established plants that were up to 90 cm in height. Resprouting from older stems that had been damaged or eaten by herbivores was observed (Fig. 3). The stems in larger specimens of the plant are up to 1.3 cm in diameter.



Fig. 3. Young leafy shoot of *Philotheca freyciana* at Mount Mayson, resprouting off an older stem.

The limited observations suggested that *Philotheca freyciana* was slow growing in the wild.

General observations also suggest that the plant has an extended flowering period. The plants were visited by generalist pollinators such as beetles and small flies (Fig. 4).

It was hoped the basic data might give an indication of how this plant would perform in a horticultural setting. Unfortunately circumstances only allowed for initial parameter measurements and a follow-up survey six months later.

c. Propagation of *Philotheca freyciana*

Rationale of Propagation Trials

Philotheca freyciana is a compact shrub commonly less than 60 cm in height with attractive 5-petalled white flowers which are pink in bud. It aesthetically has good horticultural potential but at the time of the study the practicality of propagation was unknown.

Vegetative Propagation – cuttings

Cutting material from the two populations at this site was collected in October 2002 and propagation trials were completed.





Fig. 4. Flowering specimen of *Philotheca freyciana*. Two species of beetles and small flies were seen visiting flowers.

Since this was the first attempt at cutting propagation in this species, a range of wood types was trialled to assess the most suitable. In general, softwood and semi-hardwood cuttings were quite successful, using standard RTBG cutting mix, following treatment with the commercial rooting hormone Clonex Purple™.

Less success was achieved with the hardwood cuttings, but this increased after wounding and treatment with the same rooting hormone (Fig. 5). Until rooting was established, all cutting material was initially held on a heat bed in conjunction with a misting system.

Rooting in some cases was well established about 8-10 weeks after initial propagation and the plants were then potted on, using standard RTBG native potting mix.

The success rate of this method of vegetative propagation would indicate that it is a suitable tool for the ex-situ management of this threatened species. The preferential use of softwood and/or semi-hardwood cuttings would probably increase this success rate and may be the optimal route for commercial production. Seasonal timing of the material may be a critical factor here.

Propagation from stock plants in the Nursery was the most successful. This would be due to the fact that the stock material was never stressed



Fig. 5. Cuttings of *Philotheca freyciana* in standard RTBG native potting mix.

and cutting material was available for propagation immediately after cutting. In the field the cutting material is wrapped in a moist bag but often it is stored in a fridge or esky for several days before it is used for propagation. Once a good stock collection was established, production would be relatively straight-forward. Most of the plants flowered in their pots within the first year of production. Four plants were planted out at the RTBG in dolerite soil and their progress is being monitored.

Seed Propagation

At this stage, seed propagation has had limited success with the main problem being the difficulty in obtaining enough material. In 2002 seed was collected from 3 locations – Cape Tourville (1 genotype), Mt Amos (3 genotypes) and Mt Mayson (2 genotypes). In total, only 17 seeds were available and in November 2002 these were treated with boiling water, and allowed to soak overnight. They were then sown in native seed raising mix and placed in a glasshouse on a hot bed.

After 10 weeks there was no sign of activity, but eventually two of the seeds germinated after about 12 weeks, one subsequently survived and continues to grow well. Clearly, much more seed is required so that further germination trials under different conditions can be undertaken.





Tissue Culture

It was possible to introduce *Philotheca freyciana* into tissue culture using standard conditions and to obtain shoot proliferation, but not to root the shoots (Fig. 6). Hence this cannot currently be used as a propagation method.

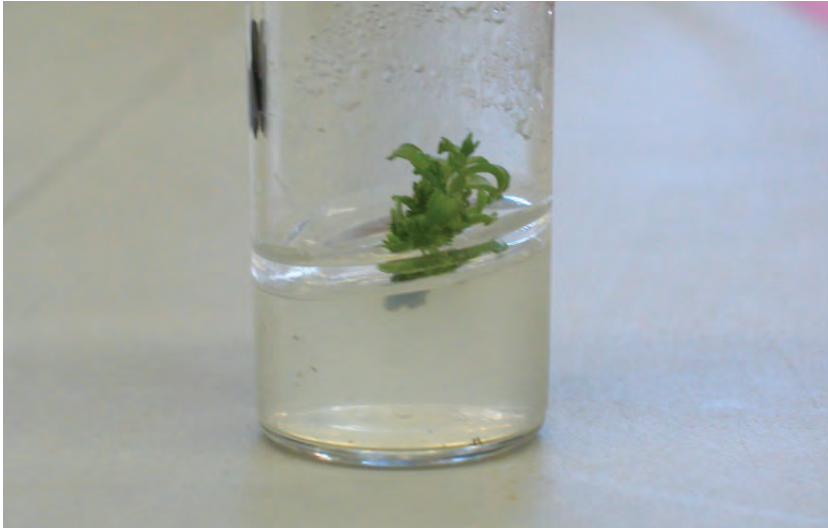


Fig. 6. *Philotheca freyciana* shoot explant.

Discussion

From the results of the various propagation methods that have been trialled thus far it is clear that from a practical point of view standard nursery vegetative propagation methods are the most effective. Initially the RTBG held 70 cutting-produced plants in pots (Fig. 7). A limited number were planted out in a garden bed at the RTBG but they did not thrive, probably due to inappropriate edaphic conditions. They were planted in a dolerite clay but their natural habitat is an open granite sand or in drip cracks in the rock.

Propagation from seed was unsatisfactory in part due to the difficulty in obtaining material in the wild. Of the two seeds that germinated one did not get past the first leaf stage but the other survived. It may now be feasible to collect seed from nursery stock plants as it is not justifiable to continue to collect from a small population of wild plants. Although shoot proliferation was achieved in tissue culture, the stock was from a more restricted range of genotypes and while suitable for horticultural production, a wider range of genotypes would be needed

for conservation outcomes. However, the initiation of root development is yet to be achieved. Although experimental work is continuing if this proves unsuccessful then further propagation should be undertaken using standard nursery procedures. If improved propagation results can be achieved it will be possible to explore the horticultural potential of this attractive species.

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Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiologia Plantarum* 15, 473-497.

Newell C, Growns D, McComb J (2003) The influence of medium aeration on in vitro rooting of Australian plant microcuttings. *Plant Cell, Tissue and Organ Culture* 75 (2), 131-142.

Endnote

The Royal Tasmanian Botanic Garden includes *Philotheca freyciana* in its major potted collections of plants listed as endangered under Tasmanian legislation. Its statement on this species is that "Our plants are currently being grown as a seed orchard to develop a seed bank collection for long-term conservation storage. By establishing a good sized seed collection within our seed bank facility we will then be able to study germination requirements and also look at seed longevity. By understanding both of these seed traits conservationists will be able to factor in the role that soil seed banks might play in this species and also under what conditions recruitment is most likely to take place." [Ed]



Fig. 7. One of the successful cutting-produced plants.





Research on eastern Australian *Persoonia*

Nathan Emery, Katharine Catelotti, Cathy Offord

Together, the three authors form the 'Persoonia team' at The Australian PlantBank, at The Australian Botanic Garden Mount Annan. They are researching the ecology and seed biology of nine *Persoonia* species to improve conservation, propagation and cultivation of these species.



Why do we conserve *Persoonia*?

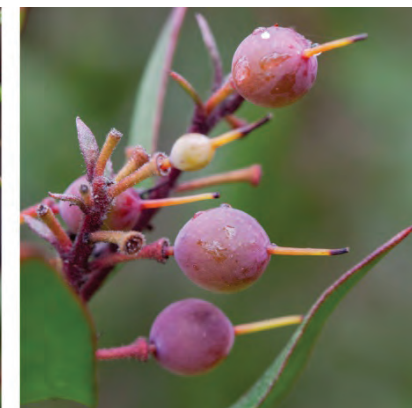
Persoonia are Proteaceous woody shrubs or small trees that are easily identified in the wild by their small, yellow hermaphroditic flowers which produce fleshy, drupaceous fruit. The genus belongs to the Persoonioideae sub-family and, unlike other Proteaceae, lack proteoid roots (Bernhardt and Weston 1996). The genus is endemic to Australia, with most species occurring in sub-tropical to temperate climates with well-drained, nutrient-poor, acidic soils common to south-eastern and south-western Australia (Weston 2003). *Persoonia* often occur in sclerophyllous, fire-prone habitats where some species, such as the threatened *Persoonia hindii*, resprout after fire, while others, such as *P. linearis*, are obligate-seeders that depend on the soil seedbank for population survival as adult plants are killed by fire. A number of species are listed as threatened, endangered or critically endangered (under the *Environment Protection and Biodiversity Conservation Act*, 1999), and are adversely impacted by a number of factors, including fire, land clearing, habitat fragmentation, grazing, slashing and predation. For this reason, scientific research is vital to provide a better understanding of *Persoonia* in order to successfully conserve these species in the wild. We can then better prioritise efforts to cultivate and propagate species for horticultural or restoration purposes.

The bright green new foliage of *Persoonia* make them conspicuous in woodland ecosystems. The foliage varies dramatically among species, some of which are sought after by the horticulture industry for their striking weeping and lush appearance. Peak flowering for east-Australian *Persoonia* can commence as early as September, with most species flowering by November. Flowering occurs for as little as one month, up to six months for some species, and is likely to be influenced by plant age, habitat quality and local climate. Despite having hermaphroditic flowers, previous research has shown that a number of species preferentially favour out-crossed pollen.

This article introduces some of the main issues associated with *Persoonia* conservation and horticulture, specifically highlighting the difficulties with propagating and cultivating plants. We foresee a focus on seed biology to be of greater overall value for the horticultural and restoration industries. For this reason, we focus on the seed stage with preliminary observations from current research at the Australian PlantBank at the Australian Botanic Garden Mount Annan.



Persoonia hindii fruit (Photo: N. Emery)



Persoonia myrtilloides fruit (Photo: N. Emery)

Ecology informs us

For a plant to successfully grow and survive in the wild requires the appropriate combination of biotic and abiotic factors, such as temperature, rainfall, soil environment and the presence of pollinators. Indeed, it is widely accepted that these, and other factors, contribute to a species being present in one ecosystem and absent from another (i.e. species distribution). Although there have been long-term datasets gathered for some species, others only have short-term data and most have no data at all. Without getting too bogged down in a plethora





of information, the survival of most plants relies on seed production followed by environmental conditions that permit at least some of these seeds to germinate and grow.

Proteaceae are characterised with a low flower to fruit ratio. Seedling recruitment is often very low and may require an environmental event, such as a fire for recruitment to even occur. Compounding this for *Persoonia* is the long time required for a fruit to mature on the plant (between 2–12 months, depending on species). As the plant allocates most of its resources to fruit development, any physical or environmental stress during this time may cause the plant to prematurely drop or terminate fruits. When a fruit fully matures it will then drop from the maternal plant. Our preliminary evidence suggests that a fruit is not fully developed until just before it is detached from the maternal plant. Therefore, fruits that are harvested from a plant are less likely to be viable than those collected from the ground or in bags over the fruiting branches.



Collecting *Persoonia pauciflora* fruit from a 'drop sheet' (Photo: N. Emery)

The proportion of fruit that successfully mature can vary significantly between species. Across the scientific literature, reports of fruit set vary between 0% and 50% (Krauss 1994; Cadzow and Carthew 2000; Wallace et al. 2002; Rymer et al. 2006). Furthermore, fruit set also varies within a species. For example, Bauer et al. (2001) reported *P. virgata* to vary between 36% and 41%, whereas Wallace et al. (2002) later note other populations to vary between 0.5% and 49%. Recently, it was determined that fruit set in *P. longifolia* was more variable among plants within a population than between populations (Chia et al. 2015). This variation in fruit set is common throughout *Persoonia* and raises



the question of what are the main factors contributing to this? For *P. longifolia*, for example, it was discovered that fruit set was positively correlated with plant height and time since fire.

However, there are more factors that are likely to be contributing to the variation in fruit set. The example of plant height may possibly be a secondary effect of plant attractiveness to pollinators. Overall plant height, plant density and

floral display size are all known to influence the visitation rate of local pollinators of many Australian plants. In turn, the effectiveness of pollinators on the level of outcrossing in plants is dependent, in part, on the level of self-compatibility of the plant. As mentioned, *Persoonia* have a preference for outcrossed pollen (i.e. higher likelihood of fruit set) despite exhibiting a degree of self-compatibility. Indeed, native bees, in particular *Leioproctus* spp., appear to be the main pollinator of numerous *Persoonia* species (Bernhardt and Weston 1996). Bees are known to be effective pollinators as they often visit a large number of flowers. However, the degree of within-plant pollination is more likely to increase in rarer plant species or those with low density populations. This is of particular concern for *P. pauciflora* (North Rothbury *Persoonia*), a critically endangered species of around 500 individuals known to occur over a small area of 29 hectares in the Hunter region of New South Wales.

At the Australian PlantBank, we are researching the ecology of *P. pauciflora* in order to better understand the factors controlling fruit set. It is with this knowledge we can then propagate and cultivate plants more efficiently and boost current population numbers by optimising a translocation program in the wild. In order to predict fruit set in this species, an understanding of the effect of plant density, plant height and flowering display size on pollinator presence and visitation is required. In our early pollinator observations, flowers were predominantly visited by at least two *Leioproctus* spp., and the frequency of visitation was associated with plant height and floral display size. An individual bee would visit up to 60 flowers on a single plant before moving to another conspecific. This suggests that within-plant pollination is possible.

Geitonogamy is the pollination of a flower by pollen from another flower on the same plant. In some *Persoonia* species, such as *P. mollis* (subsp. *nectens*), *P. mollis* (subsp. *maxima*), *P. lanceolata* and *P. glaucescens*,





Mesh bags placed over flowering branches to exclude pollinators (Photo: N. Emery)

geitonogamy does not produce viable fruit (Rymer 2006). In this instance, within-plant pollen is wasted and geitonogamy has a negative effect on plant fitness. Other species, including *P. bargoensis* and *P. virgata*, show at least partial self-compatibility. In both cases, however, pollen from the same plant produced significantly fewer fruits when compared with outcrossed flowers. Given the number of visits to flowers on a *P. pauciflora* plant that *Leioproctus* bees visit, it is important to determine the likelihood of fruit set from both geitonogamous and outcrossed pollen. Furthermore, if *P. pauciflora* is self-compatible then this could have a negative impact on the genetic diversity of the species, and also be an important consideration for its management.

Understanding a species' ecology permits more informed decisions for translocating plants in the future, such as how many individuals need to be translocated? At what density should individuals be planted to attract pollinators? Are the main pollinators of *P. pauciflora* present in the local area? Our next step is to determine the longevity and viability of fruit in the wild. This will provide important information for storing seed *ex situ* as well as estimating the possibility of natural recruitment events.

Seed biology helps us

Aside from the source of pollen, the local environment also plays an important role in seed biology. Plant populations often display adaptations to the local environment, and these can be inherited by the seed progeny. However, some degree of plasticity exists and may result in a seed germinating outside of 'optimal conditions'. To that end, we are examining the seed biology of nine *Persoonia* species, including



several that are rare or threatened, to define the window of conditions for germination and if that window changes over time. To enable us to examine the germination temperature window of multiple species in a short period of time, we are exposing seeds across a temperature gradient table. This provides an indication of the temperatures required for germination to occur, from which more specific temperature regimes can be targeted.

Characteristic of *Persoonia* and some other Proteaceous species is the hard woody shell (the endocarp) that surrounds the seed. This shell must be cracked, weakened or completely removed in order for the seed to germinate. Studies have shown that germination can remain inhibited when 50% of the endocarp still remains attached to the seed. This could be due to inhibitory compounds within the endocarp, as was recently discovered in 17 species of *Grevillea* (Ma et al. 2015). In the lab, *Persoonia* fruit are first cleaned and allowed to dry. The endocarp is then cracked in a vice and the seed is removed and washed in a weak bleach solution before being placed on an agar medium for germination to commence. It is well-documented in the literature that physically cracking the endocarp in the lab is a risky procedure that can irreversibly damage the embryo. Despite this, cracking the endocarp in a vice remains the most effective means in which to quickly extract the seed.

The rate at which the endocarp degrades in the wild is unknown. Furthermore, whether the seed within remains viable long enough for the endocarp to sufficiently degrade in the soil is also not known. If not, then this could explain why natural recruitment events are rarely observed. *P. pinifolia* seeds, for example, were estimated by Auld et al. (2000) to have a half-life of just one year. The viability of *P. elliptica* seeds similarly declined from 39% to 5% after twelve months. Such a decline, however, is likely to be variable among populations. Seed burial trials and rapid aging of fruits will provide an insight into the longevity and viability of *Persoonia* seeds.

Coupled with the physical dormancy mechanism of the endocarp is an embryo that requires the addition of a germination stimulant, such as the widely-used gibberellic acid (GA3), to improve the chance of germination (overcoming physiological dormancy). This requirement for a stimulant may not be necessary for all *Persoonia* species, as





several species have shown no improvement to germination success when GA_3 was used. It is also likely that the effect of a germination stimulant may vary between years, depending on the conditions during fruit maturation on the maternal plant. The application of smoke water to stimulate germination has been met with little benefit over GA_3 , despite its capacity to significantly improve germination rates in many Australian plant genera. However, there may be an underlying reason for this. In a recent discovery, it was found that both smoke and GA_3 have a similar effect on the seed – both catalyse germination by opening the respiration pathway by converting oxygen within the seed. In addition, smoke was noted to catalyse the production of enzymes that stimulate the movement of starch compounds from the endosperm to other parts of the seed (Sunmonu et al. 2016). Given that *Persoonia* seeds are non-endospermic, there may be no benefit of using smoke water over GA_3 . Further testing will be required to confirm the validity of this hypothesis. It is also probable that smoke catalyses other chain events within a seed.

A holistic approach

There is a lot of information to be found in the literature on *Persoonia*. Much of this published work focuses on attempts to propagate and cultivate the plants from seed or vegetative cuttings. These studies form a more problem solving, trial-and-error approach. To truly improve how we should be propagating *Persoonia*, we first need to understand what the plants are doing in the wild. Once the main factors driving fruit set and viability are determined, we can then be more targeted in our

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approach to problem solve and prioritise how we can conserve species *ex situ* and propagate plants when necessary. In other words, we need ecology to guide conservation and horticultural practises. The research we are undertaking at the Australian PlantBank aims to unravel some of the perplexities of *Persoonia* so that we might help guide and direct managers and growers on how best to work with this iconic native plant genus.

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Unlocking germination secrets: A method for *Eremophila glabra* ssp. *glabra*

Carole Elliott



Eremophila glabra ssp. *glabra* in the mallee woodland habitat at Pulletop Nature Reserve, north of Griffith in New South Wales.

Introduction

Seed of many Australian plants can be difficult to germinate as there are numerous mechanisms that need to be overcome for germination to occur (Bell 1999). *Eremophila* species are a prime example, as many of them have several barriers to germination (215 recognised species; Chinnock 2007). These mechanisms include physical barriers, chemical inhibitors and the requirement of temperature stratification and/or light exposure regimes (Chinnock 2007; Cochrane et al. 2002a; Richmond & Ghisalberti 1996).

The physical barrier of fruit walls is considerable in *Eremophila*, as they are often very thick and woody (0.5-1.5mm; Figure 1a; Chinnock 2007). Time is needed for fruit to be exposed to natural weathering processes that weaken the walls and open the apertures of the fruit (Figure 2a; Richmond & Chinnock 1994). When the fruit wall is breached the seed is exposed to oxygen and water and, therefore, the conditions necessary for germination.

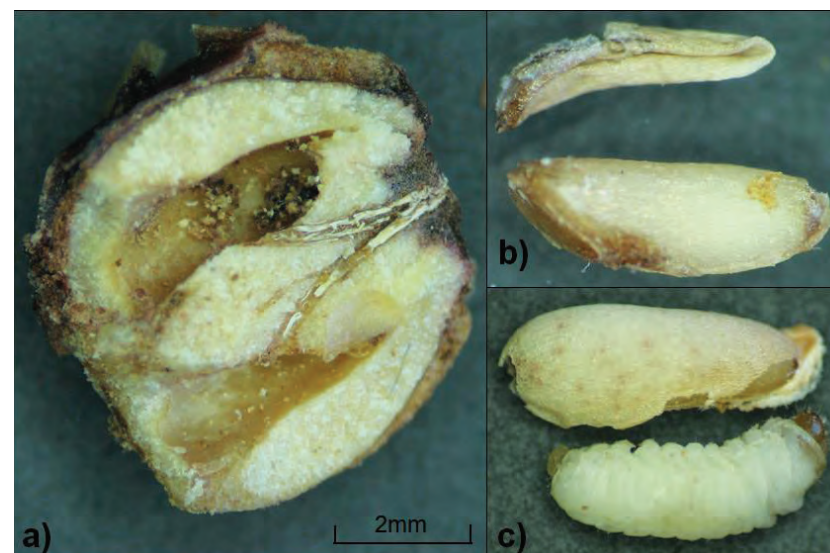


Figure 1: Illustrates *Eremophila glabra* ssp. *glabra* a) thick fruit wall and seed cavities or locules of an opened fruit; b) characteristics of non-viable seed (top) and viable seed (bottom); and c) viable seed (top) and larvae found in a seed cavity (bottom).

In addition to physical barriers, chemical inhibition may need to be overcome before seed can synthesize the hormones required to stimulate germination. These inhibitors can be found within the fruit wall (Richmond & Ghisalberti 1994) and the embryo itself (Plummer et al. 1995). Gibberellins are naturally synthesized by embryos and are primarily involved in post-dormancy metabolic processes leading to embryo growth and radicle emergence (Thomas 1992). Application of gibberellins, like gibberellic acid, provides a greater chance for seeds to start metabolizing quicker than they would normally and therefore germinating in a shorter time frame (Bell et al. 1993). In addition, compounds associated with smoke and fire have been found to stimulate the germination of many Australian species, which are often dependent on fire for recruitment (Dixon et al. 1995).

Temperature stratification or light regimes also stimulate germination and these triggers often indicate the passing of seasons, like winter or summer, where conditions may or may not be suitable for the survival of germinants. This is a prominent requirement of plant species in the northern hemisphere (Baskin and Baskin 1989), but here in Australia there is growing evidence that our species also require temperature stratification (Bell et al. 1993).

Studies of the germination requirements of *Eremophila* species have found several techniques are necessary to stimulate germination



Bird-pollinated
flowers of
Eremophila glabra
ssp. *glabra*.



(Cochrane et al. 2002b; Richmond & Chinnock 1994; Richmond & Ghisalberti 1994). In light of this previous research, I conducted a study to determine the optimum conditions for germination of *Eremophila glabra* ssp. *glabra* seed. This study was an important prelude to my investigation into the fitness of *Eremophila* seed sourced from natural populations that occurred in different situations in a fragmented agricultural landscape (Elliott 2010).

Fragmentation of native vegetation reduces the size of plant populations and increases the distance between plant populations in the landscape, which may affect the ability of populations to exchange pollen and seed, leaving a legacy of populations operating in isolation, but unable to persist for the future. The study expected populations of *Eremophila* (or indeed any other species) in large vegetation remnants to be more likely to produce seed and seedlings that were of greater fitness than populations that were in small, linear and isolated vegetation remnants. Poorer fitness of seedlings in these disturbed remnants can be due to many factors, including reduced pollinator services and therefore pollen input, or fewer individual plants to mate with and therefore an increased chance of mating with relatives (inbreeding). Determining the germination mechanisms of *Eremophila glabra* ssp. *glabra* will assist in the assessment of the effects of fragmentation on seedling fitness, but will also provide information for other *Eremophila* species that will aid in the preservation of endangered species listed under the EPBC Act 1999 (17 species; www.environment.gov.au) and allow further horticultural development of other species as spectacular garden plants.

Removing the physical barrier

The germination experiment was conducted on seed excised from mature fruit collected from natural populations in central NSW. Mature fruit was often identified as those that had changed from green to white or pale pink, dark red or for older fruit, no flesh on the fruit (Figure 2b). Fruit was dried in an oven at 37°C for a week until the flesh of the fruit had dried (a week or two in the sun would also work). Not all the fruit collected contained seed, as the species is known to be parthenocarpic, meaning flowers can produce fruit without the ovules being fertilized or pollinated (Gustafson 1942). The technique I used to extract the seed involved cracking open fruit with multi-grip pliers (i.e. adjustable). For minimum damage to seed, fruit was cracked longitudinally by applying pressure to the tip and base of the fruit with multi-grip pliers. The benefit of using adjustable pliers was being able to set the right depth of pressure needed for different sized fruit, allowing fruit to be cracked and not crushed and, therefore, reducing the risk of damaging seed. Once open, seed either fell out or was gently removed with a fine pointed needle. This also allowed the visual assessment of the viability of seed contained in fruit (Figure 1b & c). Seed was manipulated with a paint brush to avoid any damage after removal from the fruit, due to a papery thin seed coat over an oily endosperm that was easily damaged (Figure 2c). Seed can be stored in small vials or jars in a cool, dry place for up to six weeks.

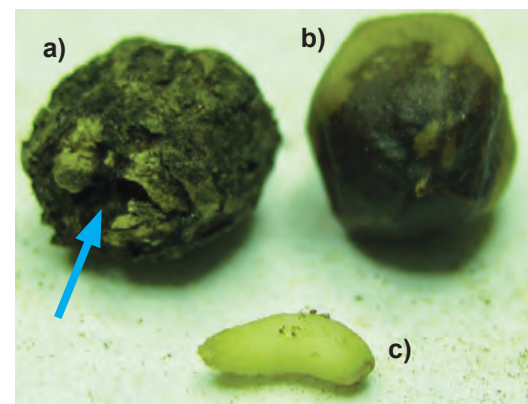


Figure 2: Illustrates *Eremophila glabra* ssp. *glabra* a) fruit that has spent several years on or under the ground; b) mature fruit that has been collected off the plant directly; and c) seed excised from fruit. The arrow indicates the apex of the fruit where a hole has formed due to weathering.

Testing chemical inhibition and temperature stratification

The design of the germination experiment involved testing the application of smoke water, gibberellic acid and different incubation temperature regimes in the stimulation of germination. I compared four treatments: 1) a control (no treatment); 2) smoke water at a concentration of 10%;





3) gibberellic acid at a concentration of 100mg/L in water and 4) equal mix of smoke water (10%) and gibberellic acid (100mg/L). Smoke water was kept at room temperature and the gibberellic acid solution was kept in the dark in the fridge while not used. There were two replicates per treatment, with four seed in each replicate. Seed were placed on wettex sponges that were soaked in the respective treatment, to allow the exchange of oxygen, for 24 hours. Imbibed seed was sowed at a depth of ~5mm in potting mix, and watered daily. The four treatments were conducted at two different temperature regimes 1) a constant 25°C day and night temperature, and 2) a 25°C day and 10°C night temperature cycle. Rotating sowed seed through a refrigerator would mimic this temperature stratification until germination, and has been done successfully (Rezl, 1996). The experiment was completed in autumn with 10-11 hours of light. I recorded their germination over the following months (Figure 3 and 4).



Figure 3: *Eremophila glabra* ssp. *glabra* seedling (approximately two weeks old) from one of the treatments.

Results

The germination response of *E. glabra* ssp. *glabra* was different among the four treatments and is presented in Table 1. Germination of *E. glabra* ssp. *glabra* occurred in all treatments and temperatures except for smoke water at constant 25°C. Seed treated with smoke water, gibberellic acid or a combination of the two germinated in 11-25 days. Seed germinated in these treatments in a shorter time frame when compared to the control treatment, which took 85-579 days for germination. The 25-10°C temperature regime had the highest germination response across all the treatments when compared to the constant 25°C temperature, except for the smoke and gibberellic acid treatment. The highest percentage of seed to germinate occurred in the 25-10°C temperature cycle, with the seed treated with gibberellic acid.

Table: The average proportion of seed that germinated (from 8 seed) and number of days to germination, for each of four treatments, across the two temperature regimes.

Temperature stratification	Constant 25°C		25°C day and 10°C night	
Treatments	Average germination	Time (days)	Average germination	Time (days)
1) Control	25.0%	85-252	37.5%	221-579
2) Smoke water	0%	0	12.5%	18
3) Gibberellic acid	12.5%	11	62.5%	13-25
4) Smoke water and gibberellic acid	25.0%	11	12.5%	11

Discussion

The optimum conditions for the germination of *Eremophila glabra* ssp. *glabra* were consistent with the expectation that these species require removal of seed from the fruit due to the significant physical barrier of the fruit wall. This study also showed that for germination to occur in a reasonable time frame and with the best success, the application of a natural growth hormone (gibberellic acid) was required to overcome chemical inhibitors in the seed. Interestingly, smoke water did not greatly influence germination. This perhaps is not unexpected, as this particular species exists in semi-arid ecosystems where recruitment in natural populations may be more dependent on heavy rainfall events and not upon fire to stimulate germination (Bell 1999; Jurado & Westoby 1992). However, this may not be the case for other *Eremophila* species (see Boschen *et al.* 2008). With regards to temperature stratification, it appears that this species had a positive response to cool night and warm day temperatures, regardless of the treatment type. This result falls within the optimal germination temperatures of other *Eremophila* species suggested by Chinnock (2007) and Richmond and Chinnock (1994) and indicates that temperature stratification is an important factor in successful germination of *Eremophila*. The germination response of *Eremophila* to these treatments supports the research on germination in other Australian native plants where not only multiple cues are required, but the cues often need to be sequential as well, to overcome dormancy and stimulate germination (Bell 1999).

The most fascinating aspect to the viability of *Eremophila* seed is its longevity. Seed removed from fruit and not stimulated by chemicals





were able to survive up to 20 months in the soil before conditions and metabolic pathways within the seed were suitable and activated, before germination occurred. This indicates the varied and small number of seeds ready to germinate at any one time in the natural environment and, therefore, patience needs to be exercised when waiting for untreated seeds to germinate. From a practical point of view of germinating untreated seeds, they can be kept at this temperature cycling for autumn/winter (the fridge) and then left outside for spring/summer conditions (but kept moist) until the next germination period, when the next round of seed has been activated by necessary sequential cues and is ready for germination. In addition, seed that was completely removed from the protection of woody fruit walls and stored in cool, dry conditions was found to still be viable and was successfully germinated after four years (Ian Tranter pers. comm.). However, there appears to be a limit to the longevity of *Eremophila* seed, with a decline in germination ability of seed between five and ten years (Richmond and Ghisalberti 1994).

In summary, the optimal germination conditions for *E. glabra* ssp. *glabra* involved excision of seed from the fruit; application of gibberellic acid and; a 25-10°C temperature cycling. These conditions were subsequently used for a germination trial conducted as part of my PhD, where 3,200 seed were treated this way and the overall germination rate was 51% (Elliott 2010).

Where to start with another *Eremophila* species? Understanding the plants natural environment, basic biology and starting with mature, viable seed will give you a head start. Based on the location and environment that it occurs in, decide what time of year (winter or summer rain?) the species is likely to germinate. When the species flowers (i.e. spring) and produces mature fruit (i.e. late summer) may indicate what time of year the seeds were intended for germination (i.e. winter). Determine the average maximum and minimum temperatures at that time of year for that location and decide how you can mimic these (i.e. sow during winter or mimic winter temperatures). Trialling certain treatments, as seen with *E. glabra* ssp. *glabra*, may also yield greater germination success. Removing seed from fruit may be a suitable start for increased germination for many *Eremophila* species as it is a major barrier to germination. For chemical triggers of germination, smoke water and gibberellic acid can be available from specialist nurseries, native plant groups or pharmaceutical companies. However, *Eremophila* germination research has highlighted that many species may have their own set of unique germination cues, so be persistent.



Figure 4: *Eremophila glabra* ssp. *glabra* seedlings (approximately eight weeks old).

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What really causes Mundulla Yellows?

Jim Barrow

Jim spent nearly 40 years working for CSIRO and “retired” as a Chief Research Scientist in 1992. Post retirement he continued his scientific work but also devoted time to his wildflower interests. He is a past president of the Wildflower Society of WA and recipient of the Wildflower Society Award.

Mundulla Yellows (MY) is a serious dieback disease of eucalypts and other native plants. It was first recognized in the late 1970s and named after a district in the south-east of South Australia. It is characterized by a yellowing and slow dieback of the branches of the plant. Usually this is more marked on one side of the tree or even on one branch. The veins of the leaves remain green but the areas between the veins go yellow (inter-veinal chlorosis). The leaf margins are distorted and there are brown spots on the leaves. Eucalypts respond by activating dormant buds and starting new shoots below the damaged part, but these also yellow and die. Eventually the whole tree dies. The disease occurs in disturbed areas, along roadsides, drains and powerlines and sometimes in parks. It has not been observed in undisturbed bushland.

Three causes have been put forward: it is caused by a biological pathogen; it is lime-induced chlorosis; or it is caused by inappropriate use of herbicides.

Biological pathogen

The symptoms and the spread of the disease led pathologists to suspect that it was caused by some infectious agent. However, teams led by Joanne Luck from Victoria (1) and by Dagmar Hanold from Adelaide (2) have been unable to associate any pathogenic organism with the disease. These teams include some of Australia's most eminent plant pathologists. If they couldn't find a pathogen, it is a fair conclusion that none exists. A further reason for being sceptical of this explanation is that many plant pathogens have quite a restricted range of hosts whereas MY occurs in at least 87 species of plants encompassing 17 genera and several families.

The Hanold team reported some observations that are relevant to the other proposed causes of the disease. For example, they report symptoms of MY on Jarrah (*Eucalyptus marginata*) in soils with a pH (CaCl₂) of 4.7; on Gimlet (*E. salubris*) at pH 4.8; on Flooded Gum (*E. rudis*) at pH 5.3; and on River Red Gum (*E. camaldulensis*) at pH 5.4. These observations are important. They were made by scientists skilled at recognizing MY and on trees growing in acid soils for which

lime-induced chlorosis is highly improbable. They also include a picture of a Sheoak (*Allocasuarina fraseriana*) tree growing in urban Perth and showing MY-like symptoms. This species is well adapted to alkaline soils and its range includes coastal calcareous dunes. It is not a species on which you would expect to see lime-induced chlorosis.

The Hanold team also showed that they could induce symptoms in healthy trees by grafting patches of bark taken from sick trees onto them. In contrast, the Luck team took cuttings from sick trees, removed the leaves, and grafted them onto healthy stocks. The growth from the grafts and from the stock was healthy. As we shall see later, these results are not inconsistent with the herbicide hypothesis.

The Hanold team discount the lime hypothesis because they observed symptoms on trees growing in acid soils. The Luck team gave it more weight because they followed their study with one in which they investigated the effects of pH.

The Hanold team state that they have observed symptoms on sites with “a recorded history of no herbicide sprays for at least 30 years”. Given that herbicides can be applied in little more than a few minutes, and that several different organizations are responsible for various roadside installations, it must be difficult to be certain that none had been applied. The Luck team merely mention the herbicide hypothesis.

Lime-induced chlorosis

Iron oxides have very low solubility. Hence, they persist in soils; that is why soils are often brown. On the other hand, this means that the concentration of iron in the soil solution is very low. Further, the higher the pH the lower its concentration. Many plants have evolved ways of overcoming this problem, for example, acidifying the soil just behind the root tip. Others have not and are confined to acid soils. If you plant them in alkaline soils, or add lime to the soil, they are unable to get enough iron. Iron is needed for the formation of chlorophyll; without it, the leaves go yellow: the plants have lime-induced chlorosis. Those cells nearest the veins get first crack at the limited supplies and are greenest; there is therefore inter-veinal chlorosis. (This is a bit simplified. Other things, such as bicarbonate concentration, phosphate supply and interactions with other trace elements, can also be important.)

The Victorian team, this time with Barbara Czerniakowski as the lead author, studied lime-induced chlorosis. In their first report (3) they showed that chlorotic trees responded to applications of iron and manganese. In their second report (4), they studied 40 Eucalyptus trees



and found that the sick trees grew in soil with a higher pH. They are circumspect in their conclusions saying that symptoms are influenced by factors including soil pH.

Two scientists from La Trobe University in Victoria, with Robert Parsons as the lead author, go much further. They write (5): "It now seems certain that the symptoms of MY are actually the symptoms of lime-induced chlorosis". And (6): "All of the well-documented records of MY are from sites where acidic soils have become more alkaline because of the use of crushed limestone in road-making or to the use of alkaline irrigation water; none are from undisturbed native vegetation". Thus they dismiss observations of MY on acid soils as not being well documented, a remarkable reversal of the scientific method: if the observations don't match the hypothesis, dismiss the observations! These scientists also dismiss the herbicide hypothesis because of the "fact" that farmers in the (Mundulla) region have been aware of the disorder for 70 years or more, a fact about which it would surely be difficult to be precise.

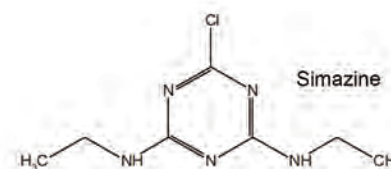
Herbicide residues

Jim McNamara from Adelaide is the main advocate of this explanation. He argued (7) that MY is caused by residues of triazine herbicides. He showed: that such herbicides were used by many different agencies and especially on roadsides and paths; that the symptoms corresponded with the known symptoms induced by triazine herbicides; and that the severity of the symptoms decreased to zero with increasing distance from sprayed areas.

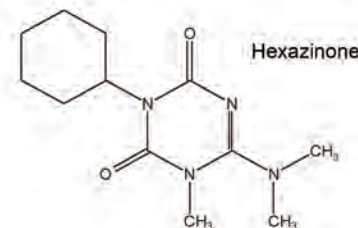
The main herbicides under suspicion are simazine and hexazinone. Both are applied to the soil, are taken up by roots and transported to the tops. Both act as photosystem II inhibitors; they cause electrons to accumulate in the chlorophyll molecules thus destroying the molecule and inducing chlorosis. If the concentration of the herbicide is sufficiently high, build-up of highly reactive molecules can destroy cell membranes causing leaves to shrivel and die.

Simazine is readily available from hardware chains and supermarkets. It is a constituent of path weeders marketed as "once a year" product. That is, it persists in soil. Hexazinone is a broad-spectrum herbicide that can control annual and perennial broadleaf weeds, some grasses, and some woody species.

The question that then arises is: can MY symptoms be associated with herbicide use?



Simazine is called a triazine because it has a ring structure with three nitrogen atoms.



Hexazinone, because of its extra oxygen atoms, is a triazinone herbicide. Hence its name.

I report here observations made in suburban Perth, Western Australia. Most of the suburbs of Perth are built on coarse sands. The present report is concerned with soils in the Spearwood system. These were formed as fore-dunes about 180,000 years ago as the sea began its retreat at the beginning of the last ice age. The surface soils are neutral to alkaline and there is free limestone in the subsoil. The local government authorities deal with run-off from roads and paved areas by leading it to low points in the landscapes. These areas are usually further excavated and the collected water rapidly infiltrates into the coarse sands and penetrates to the groundwater. These areas are locally known as "sumps". Native trees are usually retained in the neighborhood of the sumps and further screening vegetation may also be planted. The sumps are usually fenced.

In May and June 2006, more than 200 of these sumps were inappropriately sprayed with hexazinone, ostensibly to control herbaceous annual weeds. This usage of herbicide is well documented and the effects were spectacular. Observations on some of these sprayed areas form a part of this report. The remainder of the report is concerned with occasions on which I observed that herbicide had been used but it has proved difficult to identify the ingredient.

The next page shows some of the observations on hexazinone. Further pictures of these effects may be seen at <http://asgap.org.au/APOL2007/jun07-2.html>. They show that, we know: that hexazinone was applied; that it causes chlorosis; that it very quickly killed trees; and that trees that were not killed recover. Later, I show that it is difficult to prove application of simazine and that affected trees do not recover.





Left. High concentrations of hexazinone killed just about all plants within the sprayed area, including this ancient Tuart tree (*Eucalyptus gomphocephala*).

Below. Chlorotic leaves on *E. caesia* in a sprayed area. (Wembley Downs, April 2007)



Agonis flexuosa trees badly affected by hexazinone in April 2007



The same trees in March 2014. Trees that are not killed can recover.

Next, I show three examples of trees showing “classical” MY symptoms all of which are near patches of grass that had been sprayed.



A swamp mahogany tree (*Eucalyptus robusta*) in Claremont about 10 m from a patch of grass that had been sprayed with a herbicide in about May 2006. Above, chlorotic shoots from lower on an affected branch in June 2007. Right, showing chlorotic shoots in March 2008 after pruning. Note adjacent conifers are unaffected.



A young Sheoak tree (*Allocasuarina fraseriana*) near Mount Claremont library, photographed June 2002. Note the dead grass indicating herbicide use.



The same tree photographed in April 2007. The tree has since been removed.



Close up of “foliage” of the above tree (April 2007)



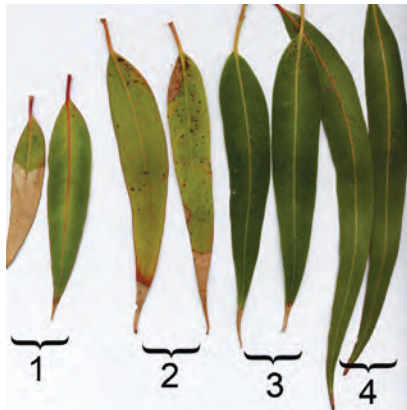
A unaffected Sheoak about 30 m from the above tree in April 2007. This tree has continued to thrive.

This species of Sheoak is well adapted to calcareous soils and is a prominent species on calcareous dunes. The only difference between these two trees is that grass near one was sprayed with herbicide.





Spotted gums (*Eucalyptus (Corymbia) maculata*) trees in Cleland St, Mount Claremont. Only the nearest 2 trees show symptoms. A small area of grass between slabs in a concrete path 15 m to the left of the first tree was sprayed with herbicide in winter 2002. Photo June 2007. In February 2016 the trees are still alive and still show symptoms.



(1) Leaves from the first tree, (2) leaves from the near side of the second tree, (3) leaves from the far side of the second tree, (4) leaves from the third tree.

Note: these trees are growing on a calcareous soil but are not irrigated.

Not all cases of chlorosis are caused by herbicide. Some are caused by high pH and high phosphate supply.



Chlorotic trees such as this Marri (*E. calophylla*) are fairly common in many public areas in Perth in which established trees are grown amongst grass that is irrigated throughout the summer with ground water drawn from calcareous subsoil. The use of phosphate fertilizers is restricted these days but nevertheless the phosphate status of the soils must be higher than for virgin soils - otherwise lawn grasses would not grow. Trees that would normally draw their summer water from the subsoil now draw it from high-phosphate soil irrigated with alkaline water. Both high phosphate supply and alkaline soil are known to induce chlorosis. Note that in the background Tuart trees (*E. gomphocephala*) (which are well adapted to calcareous soils) are unaffected.

Conclusions

I have shown here three examples of MY-like symptoms in cases for which there was a clear association with use of herbicide.

For example, only 2 of a row of 38 spotted gums showed symptoms, and the symptoms were most marked the closer to the site of application of herbicide. It is therefore clear that the symptoms were not those of lime-induced chlorosis, but were due to the herbicide. This occurrence in a few trees in an otherwise unaffected stand is typical of MY.

The symptoms mirror one of the puzzling attributes of MY: their persistence for many years. Partly this is due to herbicide carry-over in the soil: simazine is marketed as a residual herbicide. However, when a sick tree is removed, the replacement usually thrives. Hence some of the carry-over must be in the plant. This could explain why patch grafts (2) transfer some of the symptoms.

It is fortuitous that in the three cases described here I was able to observe that herbicides had been applied. There must be many cases in which the sprayed grass has long since disappeared but the effects on the trees persist. Even so, not all cases of chlorosis are caused by herbicide. There are two diseases: one caused by inappropriate herbicide, the other nutrition induced. Which is the “real” MY? Make up your own mind! But let’s stop trying to force two feet into one boot and instead talk about herbicide chlorosis and nutrition chlorosis. And wouldn’t it be nice if someone would test the herbicide hypotheses with a controlled experiment.

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Ferns and their ancient ancestors

W.B.Keith Holmes

Keith Holmes grew-up on a small dairy farm amongst the Hawkesbury Sandstone wildflowers just north of Sydney. During WWII at the then one-teacher Berowra primary school, the elderly teacher Mr Dawson gave him a lifelong love for nature - especially native orchids and fossils. Later Keith was one of the early members of SGAP. While beef-cattle breeding at Wellington in central-western NSW he was involved with the early days of Burrendong Arboretum. He became a Trustee of the Arboretum and also of the Mt Arthur Reserve. As a member of the Bodangora Landcare Group he planted thousands of trees on the almost totally cleared farmland of the district. As well as studying and growing extant plants Keith made large collections of fossil plants. He has published over twenty papers in scientific journals describing many previously unknown fossil plant species of Permian, Triassic and Miocene ages.

Whether we live in Fernbank, Ferndale or Fern Gully, or in the Otway Ranges of Victoria (see cover of Spring 2014 issue of *Australian Plants*), the dense suburbs of Sydney and visit the moist gullies of Royal National Park or Kuringai Chase, or drive through moist sclerophyll forests, or wander through relic rainforests from Tasmania to north Queensland, or be a frustrated farmer with fields of bracken, we can all marvel at the fern life that has descended from its ancestors of many millions of years ago.

Ferns are living fossils and may be compared with ginkgos, cycads, dawn redwoods and the Wollemi Pine. Ferns are among the most ancient of land plants growing on Earth. They survived the two great mass extinctions - the End-Permian Extinction (252 million years ago) that saw the demise of over 90% of living organisms and the End Cretaceous Extinction (65 ma) that saw the end of the dinosaurs - ferns must be tough!

Today, throughout the World there are approximately 12,000 species of ferns in ca 400 genera and taxonomists are still arguing on their correct classifications. Ferns occupy a great range of habitats ranging from tropical to high latitudes, from seacoast cliffs, mangrove swamps, sub-desert scrub to high altitude and arctic situations. They may be terrestrial, epiphyte, lithophyte, scrambling or climbing.

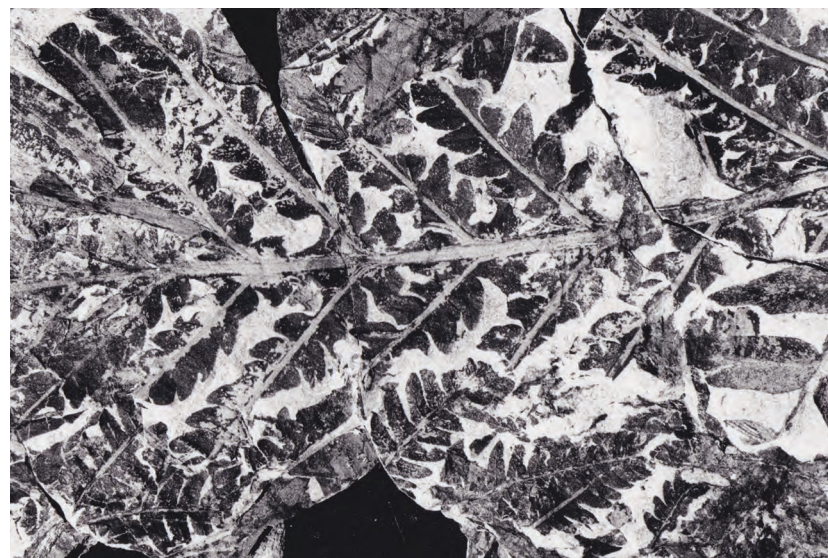
Fern morphology is often related to habitat and environment. Many structural variations and morphologies are associated with environmental conditions so one must be careful when making comparisons between families, genera and species.

The fern-like ancestors of the plants we call “true ferns” evolved with

a range of vegetative and reproductive features during the Devonian Period - approx. 400 million years ago. These fern-like plants varied in external appearance - some being tree fern-like with whorls of segmented fronds borne on 35 cm to 3 metres tall trunks. They are known from studies of their petrified axes and the morphology of their fronds and sporangia. They are best known from Palaeozoic fossils from the northern hemisphere (Taylor et al. 2009). The evolution of modern forms commenced around this time but probably from smaller less numerous forms rather than from tree-like forms.

The oldest extant family of tree ferns - the Marattiaceae - in the order Marattiales, has survived from the Carboniferous Period (ca 350 ma.) to the present day. They were important members of the vegetation in the great coal swamps of the Carboniferous in the northern hemisphere where they grew to a height of 10 metres. They differed from living species in two features; by their smaller ultimate foliar segments and their sporangia were rounded rather than elongate. Marattiaceous ferns persisted through geological time and are represented in Australia today by *Angiopteris evecta* which has been recorded from northern NSW, north through Queensland and in to Malesia.

The Order Osmundales can be traced back to the Late Permian where they are known from petrified stems and from the Triassic with indisputable foliage remains. In Australia today the family is reduced to



Nymbopteris uncinatum Middle Triassic Nymboida CM





two genera and two species: the widespread King Fern, *Todea barbara*, occurring in moist conditions along the east coast of Australia, NZ and southern Africa, and *Leptopteris fraseri* occurring in wet places in cooler rainforests along the NSW coast and Tablelands and in southern Queensland.

Other fern families known from their fossil records include the Gleicheniaceae from the Upper Carboniferous; Dicksoniaceae, widespread during the Jurassic; Cyatheaceae, treeferns to 20 metres tall, from at least the Jurassic and Polypodiaceae from the Upper Cretaceous.

Ferns diversified greatly in the Mesozoic Era and by the end of the era many forms had evolved comparable with modern families. During the Triassic and Jurassic Periods, while woody gymnosperms mainly formed the tree cover, ferns were probably an important ground cover. The evolution and diversification of the angiosperms from the early Cretaceous Period (ca 100 ma) brought a remarkable change to the World's vegetation. The establishment of new plant communities and the competition from the angiosperms would have caused many ancient ferns to become extinct, change habitats or migrate to form the World's fern vegetation as we know it today.

In Australia numerous fossil localities have yielded well-preserved specimens of ferns. The book "*Greening of Gondwana*" (White, 1998) illustrated beautifully the geological history of our plants. Cores from widespread deep drilling have produced an excellent record of fern spores.

From my own interest in palaeobotany over the years my family and I have made extensive collections of fossil plants that have included many ferns.

A locality at Cooyal near Mudgee in central-western NSW on the western margin of the Sydney Basin yielded a rich Late Permian swamp flora composed of 99% *Glossopteris* leaves (the main source of our great Permian coal measures) and some rare specimens of horsetails and ferns (Holmes, 1995). A fern was described as *Cooyalopteris dimorpha* based on an almost complete tripinnate frond with both sterile and fertile pinnae. Its taxonomic position is uncertain due to the poor preservation of the sporangia.

The great End Permian Extinction Event (252ma) caused the extinction of over 90% of earth's living organisms. However, over the next few million years the few marine and terrestrial survivors multiplied and diversified. The Nymboida Coal Measures at the southern margin of the



Osmundopsis scalaris (Osmundaceae) with fertile and sterile fronds Middle Triassic Nymboida CM

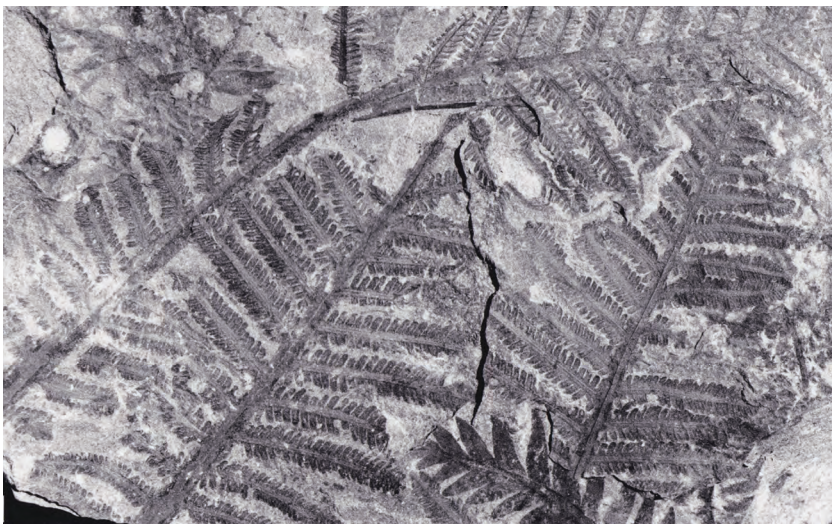
Clarence-Moreton Basin, south-west of Grafton in northern NSW, dated at ca 227 ma, were among the first commercial coal seams formed following the Extinction. Based on my extensive collections made over thirty years from two Nymboida localities the flora is well documented in publications of the Linnean Society of NSW.

The collections revealed that true ferns had now diversified into many forms, some of which are direct ancestors of today's ferns. Material from two quarries revealed sedimentary layers of conglomerate, sandstone, shale and coal seams that indicated they were laid down on an extensive flood plain. Each horizon represented a flood event and a particular facies that existed through a short period of geologic time. The floras from successive horizons may have represented differing habitats and resulted in the diversity of the preserved flora.

Ferns and fern-like fossils comprised 23% of the catalogued material. In two papers (Holmes 2001, 2003) I described the rich fern flora that ranged from tiny forms a few centimetres in height to large ferns with fronds equalling in length those of the largest extant tree ferns. Other major components of the flora were conifers, cycads, ginkgos and pteridosperms (fern-like plants bearing seeds).

The first paper described ferns preserved as fertile fronds and fronds closely associated with fertile fronds of known affinities. The Marattiales were represented by eight taxa, the Osmundales by two taxa and the





Cladophlebis tenuipinnula Middle Triassic Nymboida CM



Cladophlebis retallackii Middle Triassic Nymboida CM



Asterotheca nymboidensis (Asterothecaceae) Middle Triassic Nymboida CM

Filicales by three taxa and one taxon of uncertain position. The second paper described fern-like leaves without affiliated fertile material. Twenty three species in 12 genera were listed as morphotaxa in Order and Family Incertae Sedis.

The beautifully illustrated book “*Time on our Hands*” by Mary White (White, 1991) described remarkably preserved and petrified plant remains from the 165 million years old Jurassic Lune River Gem Beds of southern Tasmania. Fern petrifications included two species placed in the Osmundales and two in the Cyatheaales. Several rare ferns were recorded but not described.

Fern enthusiasts, when you gaze in admiration at your fern collections, or preferably at ferns in their natural habitat, please remember they have been around hundreds of times longer than the human race!

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