

# Isopogon & Petrophile Study Group

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# Seeds, fruits & hairs

Have you ever noticed the hairs spreading out in a halo from the fruit of isopogons and petrophiles? The arrangement of hairs helps distinguish between species and genus. They have an important role in seed dispersal. In this issue, we explore the seed ecology of isopogons and petrophiles.



Petrophile biternata on the Coorow-Greenhead Road WA, one of our profile species this issue. Note the red new leaf growth.

Back issues of the *Isopogon & Petrophile Study Group Newsletter* are available at <u>https://anpsa.org.au/newsletter/isopogon-and-petrophile-study-group/</u>

#### Exchanging cuttings & seed

This is a way to share propagation material between study group members. All States apart from Western Australia allow material to be mailed from NSW. If you would like to be sent cuttings/seed (may vary for seed-only requests):

- Email us to check that material is currently available. NB: cuttings are more plentiful than seed. (isopetstudygroup@gmail.com).
- Once availability is confirmed, purchase a prepaid EXPRESS POST satchel from Australia Post (Small \$12.95 or Medium \$17), selfaddress it, put in an envelope and send to: Isopogon & Petrophile Study Group

#### PO Box 291 ULLADULLA NSW 2539

- We will then package up your cuttings/seed and send it back to you *Express Post*.
- 4. An email will be sent to you on the day the package is mailed so that you can be ready to propagate as soon as the parcel arrives!

Species currently available are: **Isopogon** – anethifolius, anemonifolius (1.5m or 0.3m size), axillaris, 'Coaldale Cracker', cuneatus (shrub or dwarf coastal form), dawsonii, divergens, dubius, formosus, latifolius, linearis, mnoraifolius, nutans, panduratus ssp. palustris, spathulatus, 'Stuckeys Hybrid', trilobus **Petrophile** – glauca, linearis, pedunculata, sessilis, shirleyae, teretifolia

We need to expand the available species list to include all species growing in members' gardens. If you can provide material from other species, please let us know so we can add them to the list.

#### **IN THIS seed ISSUE**

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> PROFILE – <u>Isopogon ceratophyllus</u> PROFILE – <u>Petrophile biternata</u>

Seed characteristics Nature's seedbank Seedbank dynamics Seed release triggers Seed dispersal Germination Seedling fitness/vigour Growing a new canopy seedbank

Financial report

#### Dear members

Thank goodness La Nina has been declared over, but here on the east coast things have until recently remained hot and steamy. Less rain in total, but still making its presence obvious! Up to the end of March this year we had only a third of the same period last year (434 mm vs 1,286 mm) but as they say, it's the la Nina you have when you're not having La Nina! We are still losing shrubs but many plants are appreciating some more sun and are putting on plenty of growth.

This edition is all about seeds. What a surprise you might say given that in the study group we have been turning to cuttings and grafting for propagating isopogons and petrophiles. We've all been frustrated with low germination and damping off. We recently collected a dozen or so cones of *Petrophile pedunculata* near Bomaderry Creek Nowra – after a few weeks, all the cone scales opened but all we found was a grand total of two fruit!!! However, seed is the key to reproduction and is fundamental to conservation. We need to properly understand and document how seed works in relation to these genera. Identifying the natural processes also helps us to better focus our propagation efforts.

We have scoured scientific and other literature to bring together existing knowledge on the seed ecology of isopogons and petrophiles for the first time. We explore how the natural seedbank in the bush works – from ongoing deposits and losses to the process of release (seasonal, sporadic or one-off) followed by dispersal, germination, and development of new seed banks. We also publish some new information, report on trials, and reveal how the experts do it. We also go behind the scenes at the WA seedbank for some useful pointers. Finally, we have some useful suggestions on how you can do your own checks on seed viability. This is really important if we are to collect meaningful information about germination.

Our profiles this edition are two very different yellow-flowering species. *Isopogon ceratophyllus* is the only species found on the south coast of South Australia and Victoria. It's a compact, sculptural plant that would be a talking point in any garden. Phil loves these prickly ones and the flowers are very attractive, with an arresting combination of bright yellow and red. *Petrophile biternata* is much less common, an interesting species from the west with beautiful prominent yellow flower heads, bright green cone scales and propeller-like leaves.

Catriona and Phil

### From our members

Karlo Taliana, Georges Hall NSW Thanks for another great newsletter ...I can't imagine how much effort goes into each issue. I remember growing *Isopogon prostratus* a few years ago...I think I may have picked it up from Tony Henderson ...and I can't recall whether it was grafted or not ...but it lasted around 3-4 years and eventually the branches died off one by one in a short space of time (see right). To be honest, I'd forgotten all about this species until reading this newsletter.

#### David Lightfoot, Melbourne VIC

Thanks for the latest NL. A bumper edition!



I am growing Isopogon prostratus in a shady and reasonably dry location in my garden. It is doing okay, but not going crazy despite some good rain over recent weeks. It currently has 4 flower heads with two just starting to come out. I think I purchased it from a local native nursery many years ago in a 6 inch pot. I have not taken cuttings from it, but will after flowering.

#### Paul Kennedy, Elliminyt VIC

Just to let you know that our Isopogon formosus died after the big wet. It was eight years old and had grown to a large plant about a metre square which we lopped some 500mm off the top each year to keep it under some growth control. It always flowered very well. Now looking around for a new one. Has been very dry here but 44mm in one fall in mid February has kept the bigger plants looking OK.

#### Mike Beamish, Boolarra VIC

In the last newsletter I was complaining about my little *Petrophile canescens* neither growing much nor flowering; wandering past the plant a couple of weeks later, lo and behold, flowers! Shows how much attention I haven't been paying! They weren't very spectacular, just 3 tiny little cones hidden in amongst the foliage, and they only lasted a few days. By the time I got the camera out they were finished, but at least they were there. Here's hoping they'll put in a better, more prolonged appearance next season.

#### **Chris Clarke, Melbourne VIC**

I wonder if anyone is growing Isopogon baxteri? I took this photo in the Stirling Ranges in WA in 2017 on a botany trip with the Friends of the Australian Garden at Cranbourne. When I loaded it to iNaturalist it was the first observation of the species on that platform. The flowers have such a beautiful drooping form! Elliot and Jones say it is not common in cultivation and grows well from cuttings. It was introduced into England in 1831.



#### Liesbeth Uijtewaal, The Netherlands

I did a presentation on Australian flora last Friday and the audience was absolutely flabbergasted, they never knew the flora was that special. Isopogon formosus especially (picture from my own plant) stunned them.

I seem to recall I mentioned that P. serruriae (pictured right) and axillaris were happy but not budding up yet. I was wrong! I must have looked at the wrong place (at the top, silly me), since now I noticed loads of advanced buds on most of my plants. In the leaf axils, I should have known. All 8 serruriae seedlings and 4 of 5 of my axillaris ones carry quite a few promising buds. The seeds were sown in January 2021 so, the plants are 26 months old now. Excellent! ... they're rather tall, I'll give them a prune after flowering. They'll get a larger pot soon, too.

#### Tony Cavanagh, Ocean Grove VIC

In the APS Victoria bulletin (Growing Australian March 2023), Liz and Tony Cavanagh reported on the effects of too much rain in their garden resulting in the loss of around 30 medium to large shrubs that had died, some surprisingly among other shrubs which were not affected. Among the unfortunate correas, westringias, prostantheras and ixodias were several *Isopogon dawsonii* and *I. dubius*.

'We have an acre garden of shallow clay loam raised beds over buckshot and clay, and our oldest plants are over 40 years old. The soil is relatively dry and the land is relatively flat. After the wet winter, we received 140 mm in October (nearly three times normal) and 122 mm in November. What characterised these rainfalls was very heavy overnight falls of between 33 and 51 mm on five occasions during these two months. As a result, we were flooded from neighbours on the northern and eastern sides, and many of our losses occurred along boundaries which are usually dry and receive no watering.' Tony adds: none of these plants was grafted, it was so surprising how many died and more especially how one or two plants growing among others just died and the other plants were not affected. It certainly seems that roots of some plants just rotted. I am hoping that we don't have any more problems.

#### Darren Allen, Pokolbin NSW

I have seen a number of your presentations on grafting that are published on various platforms, and I have had an interest in native plants and propagation going back to the 1980's. I have had some minor grafting successes with Verticordias given to me by the late Max Hewitt in the 90's when I was involved with the Verticordia study group. A bushfire wiped out the garden in 2002 and took out around 800 species, many of them unnamed species that Max and his mate Ted Newman collected on their travels to WA. I took some time out from the garden for a while (10 years) and having recently retired, I have the time to continue my interest in growing and propagating. More recently I have been experimenting again with grafting with some success with a few Eremophilas, failures with Banksia, Grevillea and some of the Myrtaceae. Your presentation has inspired me to push on, and I have adopted some of the practices that you have mentioned.

I've always had a liking for Isopogon, but the garden conditions here have generally not been friendly to them. The soil is great for Shiraz (3) but a bit heavy for a lot of the natives, so raised beds and organic matter additions are necessary. Even the local I. anemonifolius struggles here, I have tried a few others I. formosus, I. dubius (clearly a masochist) and a few hybrid pink flowered species but they don't last more than a year. I think the key will be finding the right rootstock ...I would be very interested in getting started with some cuttings of Coaldale Cracker, I've tried to buy it locally without success. I read Phil's post in the newsletter about going back to grafting onto struck cuttings, so maybe I can get them started and have some ready to attempt some grafting in spring or even right away with some cutting grafts if you think it is not too late in Autumn? I've followed Phil's tutorial, but still the only real success I've had with cutting grafts is with Eremophila. Pods and various mixes were used with comparative success, small numbers only.

I'm still working out what works in my latest propagating setup, mixes/pods, mist/no mist/capillary beds, diluted bleach dip or not, clonex/ezi root/honey/nothing. And fungicides, I've tried Phosacid, Eco fungicide, bi carb, milk, tomato dust with no definitive favourite or silver bullet? I would like to focus on trying to graft and grow some of the species from WA that I've failed with on their own roots over the years, so I'll send a satchel this week and be guided by you on what to try. Whatever you think the group can benefit from. I was excited about what you are doing when I stumbled across your tutorial, as you said information has been hard to come by for a number of reasons, but I like the sharing aspect of it. Looking forward to contributing to the study and information building around I's and P's, and native plants generally.

### Isopogons & petrophiles in Armidale

#### John Nevin

Following the departure of the drought, the deluge and COVID lockdowns, our local APS group has resurrected an interest group in grafting. This was aborted with the onset of COVID but society is now more back to normal. We meet once a month on the first Sunday of the month and the location is rotated between the homes of those interested.

Over the years I had tried grafting but found the process too difficult when you had to have available suitable root stock of potted plants. Phil and Catriona had done a workshop for our group and introduced us to cutting grafts. This has revolutionised our approach to grafting and made it so much easier.

Currently our group has a diverse group of plants on which we are trying grafting. Maria Hitchcock is trying to graft Waratahs as she has had the experience of most of us that they are a very difficult group to grow. Others are grafting *Eremophila* and *Correas* using *Mypoporum* or *Correa glabra* as the rootstock.

I have been interested in trying to graft Western Australian Isopogons onto East Coast *Isopogon* rootstock (*Isopogon* 'Coaldale Cracker'). This has been made easier with Phil providing cutting material through the post of WA plants that he has growing. With *Isopogons* and *Petrophiles* I have found it very hard to access material through the nursery trade, even of East Coast plants. The other thing that I am trying is to try grafting difficult to propagate *Phebaliums* and *Leionemas* onto rootstock of plants from those genera that are easy to propagate from cuttings. This is largely trial and error (more of the latter) but I hope to pick the brain of the expert in plant DNA at our local university to see if that will provide clues as to rootstocks that are most compatible.

So far I have grafted *Isopogon divergens*, *spathulatus*, *linearis*, *axillaris*, 'Stuckey's Hybrid', *dubius* and *nutans*. I have used 'Coaldale Cracker' as the rootstock. This is a hybrid of *Isopogon petiolaris* and *mnoraifolius* from Coaldale near Grafton. It has proven easy to propagate and very hardy as well as putting on a great flowering show in Spring and Summer. It is a bit early to assess how successful I have been.

As a matter of interest, I did a survey of my garden to see just which of the *Isopogon/Petrophile* group I was growing. The *Isopogons* that I have are *anemonifolius, anethifolius, dawsonii, mnoraifolius, petiolaris* and 'Coaldale Cracker'. In the past I have had *fletcheri*, 'Stuckey's Hybrid', *prostratus* and a couple of WA ones but have been unable to sustain them long term. In the *Petrophile* group, I have growing *sessilis, ericifolia and canescens*. I have had very little experience growing from seed with my only example being *Isopogon dawsonii*. This was a very slow process, but the plant is now 40 years old and three metres tall.

### Some reflections on seed propagation

#### **Mike Beamish**

On the topic of seeds, I've sown quite a bit, several different species, over the last few years without much success, germination rates 0-1%. I reckon the main factor for this has been the age of the seed, much too old. On 3 occasions when I have used fresher seed, less than 6 months old, germination has been much better, in the range of 50-80%. The 2 species involved were *P. pulchella* (2 batches) and *P. drummondii*. My difficulties begin in the next stage of the process, pricking out and potting up. Granted, my propagation facilities are very basic and the plants do it hard, but the seedlings aren't continuing to thrive and usually stagnate in their growth or senesce completely. Of the 48 seedlings of *P. pulchella* potted up most recently, only 12 are still alive. Obviously, this is the area where I need to improve.

#### **Margaret Pieroni**

I had a really good germination of *P. helicophylla* about 20 years ago and had two plants growing well that I brought down to Denmark. They thrived and flowered well for several years before dying. I was surprised that they tolerated the clay/gravel for that long. Since then I have sown more of the seed a couple of times but without any germinating. Does the seed need to fresh or was it just beginners luck? About three years ago, two seedlings of what turned out to be the Isopogon from the Frankland area popped up in a seed tray. I hadn't sown it in the weeks or months prior but rather about 12 months before. This has occurred before with a couple of grevilleas so, when none of the seeds of *G. trifida* that I sowed a year ago germinated, I left the soil in the tray in the hope that they might germinate after 12 months and, sure enough, the first one popped up yesterday, just as we've had a little bit of rain. The moral of the story is: Don't give up on germination until at least 12 months have passed.

### Seed studies in Holland

#### Liesbeth Uijtewaal

It was a nice challenge to extract [isopogon] seeds! Like digging for gold....it is SO much easier to collect seeds from Petrophile! I noticed the dangling seeds outside the cone last summer - helps with wind dispersion but possibly animals might carry the seeds in their fur as well, away from the parent? You simply pick them off the cones. I did that with P. linearis and P. teretifolia. Loads of seeds pushing themselves out of the cone! Easy collecting, that is.

I examined a cone of *Petrophile linearis*, pulled one of the fruits out to see how it sits within the cone (with some difficulty hence the broken bract) and took photos of the process. The first photo (right) shows the starting point.



In the second photo (right) the seed is pulled out a little showing the bare side, the hairy surface is on the inside. In the third photo (far right) the seed is out albeit still somewhat attached to the cone, right in the middle of it.

What I find, in general, is that I's and P's



germinate after some 3 weeks from sowing over a fairly short period of time, one week maybe. After that no more germinating seeds were found. It turns out I've always applied smoke. In general it was Kirstenbosch smoke discs or ashes (from a friends' fireplace) or smoked vermiculite.

<u>Burning trial</u>: Your question re hairs on the fruit got me thinking, I felt they'd be highly flammable so, a protection from fire doesn't sound likely in my humble opinion. I figured that the hairs, being flammable, might encourage the seed to germinate after a bush fire too, even more so than a simple dip in smoke water. So now I've got three species in my pre-germination box, one lot of each with the seed as they are, the other lots after having held the seed in the flame of a lighter (individually, tweezers are dead handy) until the hairs were burnt. Wouldn't it be exciting to have better germination with the help of a lighter? They were all collected September/ October last year.

In my imagination all lighter-treated seeds would germinate like mad unlike the 'normal' seeds. I was wrong. I trialled three species: P. linearis, I. latifolius and P. teretifolius with 11, 6 and 6 seeds respectively for each treatment which was 1) no treatment at all or 2) lightly burning off the hairs with a lighter, for less than half a second or so. I placed the seeds between two layers of moist sturdy kitchen towel in a shallow box to be able to see exactly what happened. After a week I in a drawer at room temperature I took the seeds in and out of the fridge - at irregular intervals I have to confess but at least it must have annoyed the seeds, prompting them to germinate. Three days ago I noticed that 3 of 11 P. linearis seeds had germinated, the fourth one germinated yesterday. Not a bad score for Petrophiles! It was the non-treated batch however, suggesting that smoke/burning treatment does not promote germination at all as you already predicted. Worth a try though particularly since I experimented with Telopea seed years ago: even though about the same number of seeds germinated in the end, the ones treated with smoke water germinated more evenly and quicker than the non-treated ones.

### Can you collect seed from dead plants?

I found a dead *I. ceratophyllus* today, with seed and wondered about best practice in collecting seed from the plant. It was in an area that may have *Phytothphera cinnamomi* but there's no way to know. Perhaps it was dead from natural causes. There were other Isopogon within 50m unaffected. Would you suggest it would be best to not collect seed from this plant, and only to collect from the healthiest other plants? Darcy Kane, January 2023

It is fine to use the seed off the dead plant, even if it has died from Phytophthora. You can sow the seed that is falling out by itself now. If you collect cones it is best to leave them for at least 6 months, after which the cone should start to disintegrate. It is then a matter of pulling the cone apart to find viable seeds which should be noticeably more plump than unviable ones and discard the fluff.



### Gang-gangs' petrophile passion

In the last newsletter Peter Olde reported an amazing observation by Justin and Phoenix Greener of a flock of ganggang cockatoos feeding on young petrophile cones near Sydney. It turns out that in April 2022 Tessa Barratt also witnessed this phenomenon. She reports that she has now uploaded footage taken by her partner Joe: I was the one who heard the bird's unique "creaky" voice and spotted them but my camera is meant for the very small, and is hopeless at photographing birds. Joe had my old Panasonic with him and took this footage: <u>https://www.flickr.com/photos/125933031@N05/52474295495/in/dateposted-public/</u>

Tessa adds: This is very exciting! As it happens, I am currently involved in a conservation program through BirdLife Australia all about the Gang Gang Cockatoo. Peter's report, as well as our own, would be valuable information for the team at BirdLife. I will be letting them know. I also wanted to let you know, because it is fascinating to learn about the relationship between the birds and the plant. I just went through my course notes on the known diet of the Gang Gang and nowhere is the Petrophile listed.

### Fungi and bacteria in seeds may be important for plant growth

As well as containing the embryo and nutrients required for growth, seeds may also have microbes such as fungi and bacteria that can exist as endophytes living within their host plant. The endophytes within seeds can be beneficial because they can increase plant growth. Most of the research in the seed fungal endophyte area comes from agriculture, in plants such as wheat, corn, and barley, but recently researchers turned to natural systems and examined what is growing inside banksia seeds. Removing the fungal endophytes from the seed, they identified which species they are based on results from isolation and culturing, as well as DNA sequence analysis. The results showed that the banksia seed housed a diverse range of fungal endophyte species. They discovered that these unique fungi, which had been seen growing on the outside of *Banksia* cones, were actually growing inside the seed. Further, two species growing only metres apart (*Banksia serrata* and *Banksia ericifolia*) had fungi that were very different.

One of the mysteries about fungal endophytes in seeds is how they get there. There are a few possibilities. They could come up though the soil and up through the roots and transfer into the plant tissue and into the seeds. Or they could be transferred from the previous generation. Another possibility is through pollinators, through the flower. The fungi found in the study were not present in every banksia seed so may be coming in through particular pollinators. Fungi is present in the air and it can also get into the seed that way.

The different fungi found on the two banksia species will have very different functions and contributions to their ecosystems. They may have a role in recycling nutrients in the ecosystem and in plant health. Understanding how seeds function and whether certain species of fungi in seed are critical for that species' growth is particularly relevant for conservation of species. It may help explain species decline and could improve restoration project success. This is pioneer research and hopefully will be expanded into other *Proteaceae* genera so we can discover which fungi occur in the seed of isopogon and petrophile species.

https://www.rbgsyd.nsw.gov.au/stories/2022/seeds-of-hope-for-banksia-restoration-projects

### Seed success at Eurobodalla

Dylan Morrissey, nursery supervisor at the Eurobodalla Regional Botanic Gardens (ERBG) at Batemans Bay NSW, has shared some encouraging news on germination of *Isopogon anethifolius* achieved at the ERBG nursery. He points to surface sowing, temperature and checking seed as possible reasons for their success. The ERBG even managed to germinate seed collected in 1998 (24 years old)! Here is Dylan's report.

• Seedbank number 17044. Original plant (Acc# 1582) was collected at Rusden Head 21.9.96. Seed collected from plants on-site at ERBG on 4.10.17 and stored in fridge approx. 4°C.

- Sown 8.10.17 in standard prop mix (3 sand:2 Cocopeat:1 perlite). The resulting plants were potted up on the 21.12.17 (46 plants) and again on the 16.1.18 (28 plants). The total result from this sowing was 74/114 seeds.
- Sown 26.8.22 on the surface of standard prop mix. Sown with large difference between night and daytime temperatures and left uncovered outside under 50% shade cloth and watered daily. Potted on the 3.11.22. success rate was 64/120.
- Acc# 14052, plant originally collected 26.6.07. No location or seed collection data available. Seed sown 24.11.14. potted up on 13.5.15. 24/120 (This is old prop data, and that is the extent of what I can find on this seed. Note the longer germination time; sown later (when presumably nights were warmer) and didn't germinate until temperatures began to fall again.
- Acc# 22075 (new number, it didn't have a seed bank number or an accession number on the packet). Wild collected seed from The Castle, 1998. Sown on the surface of standard prop mix on 16.5.22. potted 6.10.22.
  9/100 seeds successful.

My observations for success are surface sowing and aiming for when there is the largest difference in day and night temps. Possibly the reason for our larger than expected germination rate is that Marjorie Apthorpe checks all the seed individually by microscope before it is packaged (see Marjorie's comments below). This may help weed out seed that is not formed/pest affected/not viable.

### A few personal observations on seed quality

#### **Marjorie Apthorpe**

In 2017 I commenced cleaning seed at the seedbank at ERBG (Eurobodalla Regional Botanic Garden, south of Batemans Bay NSW). Having a dislike of machinery and a love of microscopes, my preference was to clean the (usually small) batches of seed by hand, using fine cosmetic tweezers and desk sieves. Examining seeds under the binocular microscope enables one to sort the good from the bad. Bad seed is seed that has not been fertilised and is flat or shrunken. Many seeds that would be viable are used as nurseries for egg laying by insects, and the seeds are eaten by the larvae, or by adults. With most species, bad seed greatly outnumbers seed that is worthwhile to plant, or to store in a seedbank.

Isopogon fruit releases from the cones when ripe cones are rubbed with the fingers, forming a shower of frass (packing material) with some seed mixed within it. Isopogon fruits are somewhat smaller than Petrophile fruits, but both have a dense mop of hairs. Good Isopogon fruit is intact, and plump and firm to the touch when picked up with tweezers.

Petrophile fruits tend to be held tightly in their cones, only a very few releasing from the tip of the cone by pulling on the protruding hairs. Most are held tightly within the cone segments (see right). Extracting these can require careful use of a scalpel to cut away the woody cone plates, then gently levering out the fruits. A recently cleaned batch of *Petrophile sessilis* seed at ERBG proved to be about 90% damaged by insects. Petrophile fruit is 2 to 3 mm in length, with the hairs forming a halo several mm in length around the seed. Fruit that appears to have viable seed is plump, firm to the touch, does not have holes visible, and has only small notches, or none, at the pointed end. Seed that has been eaten has cavities within, or one end visibly missing. These features would be visible with a good hand lens.





Left: P. sessilis fruits that are apparently intact.

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Right: *P. sessilis* fruits that are definitely not intact or viable.

Images courtesy of ERBG Herbarium microscope.

Of course the proof is in the planting. In 2017 I planted small batches of both Isopogon and Petrophile seed from cones obtained from ERBG. I used a sand and fine woodchip mix for the Isopogons, and a sand mix topped with wood ash for the Petrophile. The *I. anethifolius* from ERBG #1582 germinated well, and I now have three plants, including a mature bush two



metres high. The six *P. sessilis* seeds also germinated well, but quickly succumbed to rotting of their stems at the radical leaf stage.

Pollination appears to be an issue, as many of the I & P stock plants at ERBG do not appear to produce fertile cones. Nor has my large I. anethifolius at home produced viable seed. The only "pollinators" I have seen in these flowers are weevils, of which there were many, sometimes covered with pollen grains. But no seed...

### Lessons from the past: an isopogon seed germination trial

#### **Tony Cavanagh**

#### Reprinted from Australian Plants Journal, Vol. 8, June 1976

In past issues of *Australian Plants* relatively little has appeared on the genus *Isopogon*. Several species are well known in gardens, notably Isopogon formosus, and I. dubius and, in eastern States, I. anethifolius and I. anemonifolius. I. cuneatus and I. latifolius are looked on with envy by all who see them in flower. There are believed to be some 35 species, 25 endemic to Western Australia and 10 to the eastern States. Apart from the above mentioned species, the majority of the others are not available from even specialist native plant nurseries, an unfortunate situation when one considers their unusual foliage and striking pink or yellow cone flowers. Other features worthy of attention are the small size of most species, typically about one metre high and generally less than two, and the fact that many can grow in semi shade positions. In their natural habitat a lot of them grow in close association with surrounding vegetation and, on account of their size, they are often the understory. I have lost Isopogons in very hot weather in open positions, whereas I. formosus and I. dubius have both survived the same weather in their semi-shaded spots and will be flowering heavily this year.

Why, then, are *Isopogon* spp. difficult to obtain from nurseries? Part of the answer must lie in difficulty of germination of seed and, more importantly, carrying the seedlings on. Alec Blombery in "A Guide to Native Australian Plants" says: "Germination of seed is slow and often difficult, and some treatment is usually required. Propagation can also be carried out from cuttings, but is also slow." He advocates abrasion of seed with diamond-grit or emery paper followed by soaking in near boiling water. Hazel Cole, writing on "Simple Propagation", in *Australian Plants* of March, 1971, comments that she had had no success at all with *Isopogon* spp. and *Petrophile* spp. and suggests it may be better to sow in situ. A nurseryman to whom I spoke advocated germination in clay, allegedly on the grounds that many *Isopogon* spp. grew in clay soils in Western Australia. [In an update from Hazel in 1977, she notes that Isopogon and Petrophile had given good germination: 'However I have failed to keep them growing as soon as the hot weather comes. All were sown with a few seeds to a pot eliminating pricking out. The seed is grown direct without any prior treatment, pressed into the surface and covered with the same soil to a depth equal to the diameter of the seed.' From '*I Grow Proteaceae' by Hazel Cole, Australian Plants Journal, Vol. 9, September 1977*.]

In October of 1973 I planted seeds of the following: Isopogon asper, I. anemonifolius, I. cuneatus, I. dawsonii, I. divergens, I. dubius, I. formosus, I. latifolius, I. tridens and I. trilobus. The seed mix was approximately 50 per cent coarse washed sand; remainder sandy loam and peat moss. The seeds were lightly covered with about 5mm of Yates Seed Raising Mix and about the same depth of coarse gravel. The pots were left in the open and watered about five times a week unless rainfall was adequate; they were given no special protection. The seeds were not treated in any

way. All except I. latifolius and I. asper had germinated within 32 days, the shortest time being 24 days for I. divergens, I. formosus and I. dubius. In most cases the strike was better than 70 per cent.

For interest I left the pot of I. latifolius seeds, as I had been told that Isopogon spp. sometimes come up after several months. Surprisingly, some seven months later, in late April, a seedling appeared. The pot by this time had a dense, moss-like growth over much of its surface, which I was unwilling to remove in case other seedlings were disturbed; nevertheless, four more appeared and have been potted up into individual tubes. Despite a rather severe and wet winter they have all survived and should be ready for transplanting to larger pots.

With some types of seed a dormancy exists which must be broken before germination will take place. Sometimes the seed requires storing in a cool place such as a refrigerator for about six weeks (stratification); in nature, such seeds germinate in spring or with autumn rains after going through a winter. I do not know whether *Isopogon* spp. fall into this category, but it seemed rather surprising that the seed germinated in the latter part of a cold autumn and not during the warmer days of spring and summer. To check whether cold weather may have an influence, I sowed 16 more seeds from the same batch on May 18, using this time a sand/peat moss mix. The weather was quite cold, with days of minimum temperatures down to 1-3°C and maxima of 10-14°C; rainfall was scattered but heavy, with some areas of Victoria receiving record June falls. Despite all these highly undesirable conditions, the first seeds came up in seven weeks (51 days) and by mid-July 12 of the 16 had germinated.

On the face of it these results would appear to indicate the following:

- a) If the seed is fertile, many species will germinate freely in  $3\frac{1}{2}$  to  $4\frac{1}{2}$  weeks when sown in spring.
- b) For at least the following species, prior treatment does not seem necessary: I. anemonifolius, I. cuneatus, I. dawsonii. I. divergens, I. dubius, I. formosus, I. tridens, I. trilobus.
- c) For I. latifolius a period of cold weather seems to be necessary for satisfactory germination.

Perhaps the last is not so surprising when it is remembered that I. latifolius grows on the slopes of the Stirling Ranges in Western Australia. The Stirlings are up to 1,100 metres high and occasionally receive snowfalls. Seeds from the previous October-November flowering would thus be subjected to a cold winter and would, I assume, germinate in the autumn following good rainfall.

### 40 years of seed propagation: tips from Tony Henderson

Grafting isopogons and petrophiles has been a passion for Tony Henderson for over four decades. While the grafting process requires a considerable level of skill, Tony considers the propagation of petrophile stock plants from seed an equally challenging aspect of grafting. He produces isopogon stock plants from cuttings rather than seed. We have picked Tony's brains many times about how to successfully grow petrophile stocks *P. pulchella* and *P. sessilis*. While Tony is always happy to share his vast knowledge on this topic, he always expresses frustration at how time-consuming and unpredictable it is to produce stock plants ready for grafting. Variations in germination rates, losses in the potting on process and the frustratingly slow growth of seedlings are issues he deals with, just like the rest of us. But when we visit his nursery, the abundant trays of petrophile stock plants illustrate just how successful he is.

Tony has also successful raised many western isopogons and petrophiles from seed. However, like most members of the study group he finds the western species much more difficult than the eastern species. Germination rates are much lower, and they are much more prone to damping off in the potting on process.

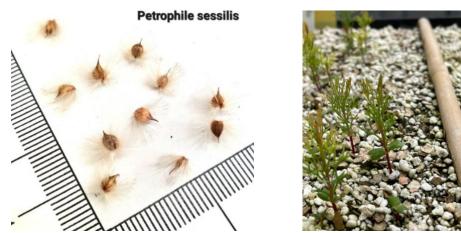
It's important for the study group to document the various methods of experts in raising isopogons and petrophiles from seed. Only then might we have the knowledge needed to replicate the success of these experts. Here is Tony's method, fine-tuned over the last 40 years.

- Seed-raising mix is 100% perlite.
- No pre-treatment Tony finds no benefit in smoke treatment or singeing hairs.
- Mature cones are chosen but not older cones as these often have borer/insect damage. Cones are stored in a shaded area to allow them to open naturally and release their fruit.

- A type of wicking method is used where the seed trays are placed in a water tray, so that water is drawn up to keep the seed raising mix moist but not too wet. The eastern species are happier with this method than the western species which can succumb to damping off.
- Germination can take more than 6 months from sowing, so patience is needed.
- Seedlings are potted up into native potting mix once they reach around 2cm in height and then exposed to the elements out in into the open.

## Sutherland Shire Community Nursery – how do they do it?

When we first visited Sutherland Community Nursery in 2021, we were stunned at the many trays of *Isopogon anemonifolius*, *Petrophile pulchella* and *Petrophile sessilis*, all grown from seed, and all in perfect condition. The Study Group has a long history of failures by members to successfully grow isopogons and petrophile from seed, so to see this demonstration of a highly successful outcome was a revelation. We are unaware of any other organisation producing isopogons and petrophiles from seed in such numbers. Being a community nursery, only local species are produced so their methods may not be applicable to producing western species from seed.



Left, training material for nursery volunteers (photo Emma Braume). Right, P. pulchella seedlings.

Horticulturalist Emma Braume has been very generous in discussing their seed-raising methods which we documented in Newsletter 30. Recently we had another chat with Emma to expand on this valuable expertise. Here is an updated summary of what is clearly a highly successful method.

- Collect last year's fruit not old cones where germinability of seed will be lower and more predated by insects. Collection of cones is undertaken around November each year. Cones are stored for 6 months in paper bags before cleaning and extracting seed.
- The only pre-treatment is smoking of seed. However, this is not always undertaken and there is no definitive evidence that this is beneficial.
- Seed-raising mix used is 50% perlite/50% vermiculite.
- Seed is sown throughout the year in cooler months where maximum daytime temperatures do not exceed 24°C. No months have been identified as better for germination, with high germination rates achieved across all the cooler months.
- Fresh seed is considered vital, with declining germination rates noted as seed ages. Seed is stored in sealed foil bags in fridges to minimise viability loss over time.
- Seed trays are sown with 3 grams of seed, with an average 300 seedlings germinating in each tray. The trays are stored in a polytunnel under misting (3 times a day in summer, reducing to only hand-watering in winter as required). The weight of the tray is monitored to ensure that over-watering is avoided. The temperature in the polytunnel is generally in the 18-24°C range. Bottom heat is used in the cooler months but is turned off throughout the warmer summer months.
- Potting mix with slow release Osmocote added is used to pot on the seedlings. These seedlings are then irrigated in the open and subject to rain.

# Secrets from the WA Threatened Flora Seed Centre

The Threatened Flora Seed Centre is Western Australia's principal seed banking facility for rare, threatened and poorly known native plant species. The seed centre aims to collect and store sufficient genetic resources from each threatened species to ensure its successful reintroduction and establishment in the wild if extinction occurs. Their processes are based on northern hemisphere storage processes developed for agricultural gene banks, tested and proven for Australian species. We visited the centre to learn about storage, germination and viability. In the seedbanking context, maintaining seed viability over time is paramount so the focus is on seed quality, storage and viability.



The process begins with seed collection. When it arrives at the centre the seed is separated from other plant material using a range of methods as required, such as an aspirator to sort the heavy (the seed) from the light, burning or even wetting and drying. Visual assessment is used to find obvious discards.

The most important step is next, drying. Seeds are kept in paper bags at  $15^{\circ}$ C in a sealed room with a dehumidifier until they get down to 15% relative humidity (tested using a hygrometer). They are then transferred to heat-sealed foil bags and stored permanently (frozen) at -20°C.



Checks for viability are undertaken when seed goes into storage and then at regular



intervals. It is vital that seed is viable to begin with, but because viability declines with age under normal conditions, it has to be checked over time. Loss of viability appears to be due to initial seed

quality. Viability is estimated using germination tests. X-rays can show whether a fruit has a seed but not whether the seed is viable. Only small samples (never more than 10%) are germinated to ensure seed stocks are adequately maintained. Smoke pre-treatment is standard (soaking fruit in smoke water); this is a 'best bet' based on previous literature rather than actual testing. Seeds are sowed in water agar (jelly) which has gibberellic acid (hormone) in the agar in sealed containers at 100% humidity and kept under artificial light in a growing room at 15°C. Seedlings should emerge by two weeks and be finished by six weeks. If not, a range of extra measures are tried including hormone, smoke in the agar or different temperatures.

How does this relate to isopogons and petrophiles? Study Group members have long wrestled with difficulties germinating seed. Here are some take home messages from the Threatened Flora Seed Centre.

#### **Seed collection**

Seeds are at their physiological peak at maturity. For this reason, we select cones on an isopogon or petrophile which have long-finished flowering and have aged to grey, usually about six months. The best time is not too young or too old, right at the time of dispersal i.e., when the cone is giving up its seed. The best quality seed is that which is easy to get or drops out of the cone. If, after a suitable drying off period, the fruit you have collected has to be pulled or persuaded out of the cone, it is less likely to be viable.

#### Each seed is enclosed in a fruit

What we think of as seeds are actually fruit; the seed is inside. The fruit is what we sow when propagating. This covering means it is difficult to check on the presence and quality of seed. Advice from the seed centre is that seed fill in these genera is incredibly low – less than 10%. This is the result of a range of factors – lack of proper fertilisation, aborted seed or insect predation, all obviously very common. Therefore, checking the fruit as closely as possible for obvious quality issues is vital if we want to maximise germination rates.

#### **Drying and storing**

While we do not necessarily have the same aims or conditions as the seed centre, the most important points to note are that reducing moisture, or drying, is crucial to maintaining seed viability, and that the age of fruit affects viability. Collecting only dry (or mature) fruit is vital. Unless the seed is being sowed immediately and it is already falling from

the cone, then further drying is required. Keeping cones as dry as possible until the seed begins to drop is sensible, preferably out of high temperatures. If you wish, you can dry it further (especially if you wish to store it for long periods of time). An effective method is to place in a sealed container with rice (dried in oven) or silica gel for a few days. The rice can be first dried in an oven. Drying progress can be monitored using a moisture meter card (right). If you wish, the container can also be placed in the fridge.

#### Germination

Few of us can provide controlled lab-like conditions for germination like the Seed Centre, involving smoke pre-treatment, agar with hormone, 100% humidity, artificial light, and a constant 15°C temperature. These result in very high germination rates in isopogons and petrophiles. Germination rates refers to actual seeds sowed, not fruit sowed. The Seed Centre checks every fruit for a high-quality seed by carefully nicking each one before sowing it.

Another method is to do a cut test on a sample. Although this destroys the seed, it will show you what to expect from your sowing and allow you to set your expectations of success.

Thanks to Dr Andrew Crawford, Research Scientist, Threatened Flora Seed Centre, Department of Biodiversity, Conservation and Attractions, Western Australia.

### How to avoid disappointment: check seed quality

There is no point wasting time on trying to germinate or store seed that is no good. Insect damaged or shrivelled fruit is unlikely to contain seed that will germinate and fruits with no seed within are useless. If the success of your planting effort is dismal, it may have been due to lack of germinable seed within the seed batch used. To facilitate good germination, you need the best quality seed.

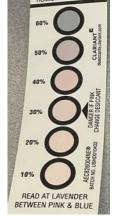
In isopogons and petrophiles, only one in ten fruits will contain an intact seed. Whole batches of fruit may have no viable seed. There is plenty of experience of this in our study group. Across three cones of *Isopogon anethifolius*, John Knight found an average of 4.8 'good' fruits per cone. Across 15 cones of *Isopogon latifolius*, Liesbeth Uijtewaal found an average of 9.3 'good' fruits per cone, varying from zero up to a maximum of 18. In *Isopogon anethifolius*, Marjorie Apthorpe has observed that cones without viable seeds seem to be more common in the nursery than the wild in her area.

With isopogons and petrophiles, we are essentially working blindfolded because the seed is concealed inside a hard, dry fruit. We plant fruits but germination refers to actual seeds sowed, not fruits sowed. This fruit casing can hide damaged or shrivelled seed, or the absence of a seed. For this reason, seed quality should be assessed in some way before attempting propagation. This makes information on germination rates more accurate, which is important for study group research.

There are some quick checks that can be conducted on fruit to determine whether the seed hidden inside is potentially viable. At a minimum, we recommend doing a visual inspection and touch test before attempting to germinate seed of isopogons and petrophiles.

#### Collection

Healthy, fat seeds are more likely to emerge from the cone. Seedbanking experts prefer to collect fruit which springs from the cone unassisted, indicating maturity and viability, rather than seed which needs to be reluctantly prised out of cones.





#### Cleaning

The first step is distinguish seed from non-seed material such as chaff, seed decoys and floral parts. Opening the cone.... Isopogon seeds straight off the cone come with packing material (chaff) and cone structures – petrophiles do not. As in nature, the whole lot can be put down for germination. However, picking out the seed is a way to do preliminary checks of viability and to accurately count the number of seeds. Right: chaff, involucral bracts, cone scales and aborted seed taken from a cone of *Isopogon latifolius*. This cone had no viable seed.

#### **Visual inspection**

Look at the fruits closely to identify obvious discards. The naked eye is usually sufficient, but you may wish to use a magnifying glass. At the Eurobodalla Regional Botanic Gardens, Marjorie Apthorpe uses a

microscope and removes fruit that is damaged, flattened, empty or predated. She notes: 'The "good" Isopogon/Petrophile fruit that I think is viable has a swollen, plump appearance. Any holes indicate insects have eaten out the insides. Quite a lot of the fruit is flattened (presumably the seed aborted or was not fertilised).' Liesbeth Uijtewaal adds: 'in fat isopogon fruits the silky 'parachute' spreads a lot further than when there is hardly any fruit at all'. Shrivelled, broken or damaged fruits are unlikely to contain viable seed. Fruits with insect frass around them should also be discarded. After a while you will get a feel for the look of the fruit and you should be able to discard any fruit that does not look full, undamaged and plump.

#### **Touch test**

An easy way to check for plumpness is to feel for it by pressing your fingers against the fruit. Simply place a fingertip over the fruit and press down against a hard surface, or as John Knight suggests, roll fruit between thumb and forefinger. Plumpness is a proxy for weight, as fertile seeds containing embryos are larger and heavier than infertile ones. John tested this theory by comparing the relative weights of *Isopogon anethifolius* fruits he had classified as fertile and those classified as infertile. Three of each type were dropped from a height of 20 cm on to a table (first removing the hairs which might assist with descent). Those fertile fell quickly, suggesting they were weightier than the infertile, which whilst not floating down, certainly hit the table slower, and without any noise.

The following tests should be conducted on a sample of fruit only as they destroy/damage the seed or may reduce germinability.

#### Cut test

Cutting the fruit open reveals whether a seed is inside and makes damage obvious. The easiest way is to cut down straight through the middle with a sharp knife. The cut allows inspection for the presence of healthy embryonic tissues. These should appear firm, plump, swollen and generally a healthy white or pale-yellow colour. Seeds containing dry, shrivelled and/or brown tissues are usually non-viable.

Although cutting destroys the seed, it is worth doing this on a sample of your fruit. There are two ways of sampling:

- 1. Take a representative sample of your seed, either by weight or by a count. A small sample (10% or less) helps set expectations for germination levels or at least allows us to interpret our results. It may also be a useful guide to what healthy fruits look like.
- 2. Tony Henderson sorts the fruit by size. Starting with the smallest fruits, he cuts each one in half and checks the seed. He keeps going until he finds a healthy seed, then sows the rest on the assumption that the larger fruits are all healthy. A drawback of this approach is that some of the larger fruits may still not have intact seed.

A riskier cutting method is where seed is carefully nicked to check for seed before sowing. The knife must avoid the radicle (root tip) at the base – the rest of the seed consists of embryonic cotyledons which may be more tolerant of damage. The advantage of this method is that it does not damage the seed if done carefully. The disadvantage is that it could be almost impossible to do accurately! Keith Alcock has reported slitting petrophile fruit and discarding many as they were empty – however he couldn't do the same with isopogons as the fruit is too small.

#### Float test

This involves placing a sample of fruits in a container of water to test whether they float or sink. Sinking fruit should be full and healthy, whilst floating fruit is likely to be empty or shrivelled. Do a cut test on some of the floaters and the sinkers to see if this is correct. The proportion of sinkers is an estimate of viability in the seed lot. You can also use a few drops of detergent to act as a wetting agent before adding the seed, stirring it in.

#### **Germination trials**

Under controlled conditions, germination is the most accurate test of seed viability. You can do as the seedbanks do and sow a representative sample of fruit to check germinability before doing any larger-scale propagation. However, we cannot often control our conditions as in the laboratory. One way to sort viable from non-viable seed used with isopogon seed suggested by David Lightfoot who tried it successfully with isopogon fruit, is based on Paul Kennedy's hakea seed raising method. This involves placing the fruit in a folded-up piece of damp paper towelling. This is then put into a sealed plastic bag and placed in a sunny spot. The fruits are periodically inspected and then potted once they germinate.

Other methods used in laboratories include x-rays to check for the presence of seed, embryo excision and staining embryonic tissues to test for biochemical activity. The most common stain used is Tetrazolium chloride (TTC).

### Isopogon ceratophyllus R. Brown, Trans. Linn. Soc. London 10: 72 (1810)



*Isopogon ceratophyllus* (common name Horny Conebush) was first described by Robert Brown in 1810. The species name is derived from the Greek *cerat*-"horn" and *phyllon* "leaf", apparently reflecting the leaves' resemblance to antlers. Brown's description used the type specimen collected in 1804 on the hillside at Arthur's Seat on Port Phillip Bay, Victoria.

**Description** –*Isopogon ceratophyllus* is a low shrub with a lignotuber. It is probably the prickliest isopogon with flower heads often hidden in a dense, impenetrable mass of rigid, divided leaves, each flat leaf segment ending in a savage point. Leaves are pinnatisect (having lobes that extend almost or up to midrib), with a petiole (stalk) 1.2-5.6 cm long, and a flat

lamina 1.8-3.6 cm in length. The leaves are sparsely villous and usually shiny, with the leaflets (arranged on either side of the stem) twice or three-times divided. The branchlets are red-brown and sparsely villous like the leaves.





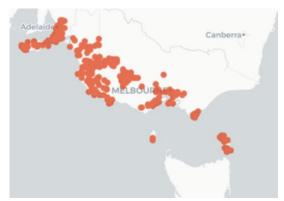
The flowers are bright yellow, to 15 mm long,

and form a stunning sight as they emerge from bright red/burgundy cone scales. Each inflorescence is closely surrounded by leaves, and leaf bases can display the same striking red/burgundy colour as the involucral bracts and cone scales. The involucral bracts are

quite inconspicuous, and similar to the cone scales. Flowers are glabrous apart from a sparse tuft of hairs on the apex of each tepal. The pollen presenter is a slightly swollen spindle shape, with a constriction and swollen ridge near the mid-

point then narrowing to a stigmatic cup at the tip. Flowering is from July to January. Cones are ellipsoidal to globose, to 22 mm diameter. The fruit is villous, ovoid and beaked, to 3 mm in length (photo right).





**Distribution** – *Isopogon ceratophyllus* grows mainly in pure sand and sandy soils in dry sclerophyll forest, woodland and heath. It occurs throughout south-western Victoria across to the south-eastern corner of South Australia including Kangaroo Island. It is the only isopogon species in South Australia. It can also be found on the Bass Strait islands Flinders, Cape Barren and Clarke and is classified as vulnerable in these Tasmanian islands.

**Confusing species** – this species is distinctive in form and prickliness and does not co-occur with any other isopogon species. Individual leaves might be confused with *I. petiolaris* as they both have a long

stalk 2-3 times as long as the divided part which also divides into three. However, *I. ceratophyllus* leaves are much more rigid and thorny. *I. ceratophyllus* is much more dense and compact. In this respect I. *ceratophyllus* is much more like *Petrophile multisecta* with which it co-occurs on Kangaroo Island. However *P. multisecta* has terete leaves.



**Cultivation** – *Isopogon ceratophyllus* strikes readily from cuttings and can also be grown from seed. It has been successfully grafted onto *I*. 'Coaldale Cracker' and *I*. *anethifolius* and appears very compatible on both stocks. In ungrafted form it can tolerate extended dry periods once established but not summer-wet climates due to high susceptibility to *Phytophthora cinnamomi*. In the garden, it is a sculptural foliage feature, its small size and compact habit highly suited to modern gardens. Slow-growing, it is suitable for rockeries and containers. Under-appreciated due to its prickly foliage and tendency to hide its flowers, the stunning colour combination of red cone scales and brilliant yellow flowers plus a prehistoric feel make it a worthwhile addition to any garden.



### Petrophile biternata Meisner, Hooker's J. Bot. Kew Gard. Misc. 7: 69 (1855)

*Petrophile biternata* was first described by Carl Meisner in 1855 using material collected by James Drummond between the Moore and Murchison Rivers in Western Australia. The species name reflects the biternate leaves (leaves dividing into three leaflets which then divide again into another three leaflets).

**Description** –*Petrophile biternata* is a small, upright shrub to 1.2 m without a lignotuber. It is an easily recognised species because of its flat, divided leaves, and viscid flowers and outer floral bracts. The flowers are a brilliant yellow or creamy yellow, viscid, up to 10 mm long, and glabrous. The pollen presenter is fusiform and covered with very short, white hairs. Flowering time is August to October. Below the flowers are prominent, often bright green, viscid, outer floral bracts (cone scales) and at the bottom of the flower head are small, brown involucral bracts. The

hard-to-see inner cone scales are acuminate (tapering) and very woolly with a glabrous tip. Cones are ovoid to 25 mm in height, while the nuts are obovate to 4-5 mm long, with a prominent, blunt beaked tip (pictured right).





**Distribution** – *Petrophile biternata* grows in shrubland in laterite, grey sand and gravel, in quartzite soil and in sandy heath. It occurs in

Western Australia north of Perth in the Moora-Watheroo and Murchison River areas and near New Norcia. Easy spots to see this species are on Tootbardi Road, Badgingarra, and on the Coorow-Greenhead Road just east of the Brand Highway. This species is possibly threatened, classified as Priority 3 (poorly known).

**Confusing species** – Other petrophiles with flat leaves dividing into three are *P. biloba* and *P. squamata*, but *P. biloba* leaves divide three times and then two again; and *P. squamata* leaves divide into three just once. Among these species, *P. biternata* is the only one with large, terminal, very prominent solitary flower heads, and also differs in

having sticky flowers and large, sticky outer cone scales. *P. fastigiata* is sometimes confused with this species but it has terete leaves and occurs much further south along the southern coast of Western Australia.

**Cultivation** – *Petrophile biternata* can be grown from seed but so far it has proven difficult to strike from cuttings. Grafting trials have been successful on rootstocks *P. pulchella* and *P. sessilis*. Ungrafted plants require very well-drained soil. In summer-wet climates of eastern Australia, grafted plants are recommended. It has proven hardy to moderate frosts, and prefers plenty of sunshine though tolerating semi-shade. The species has also shown to respond well to pruning. *Petrophile biternata* is not currently available in nurseries, a reflection of its difficulty to propagate. This is a shame as it has lots of ornamental appeal, with attractive foliage and prominent yellow flower heads in a small-medium size shrub suitable for all gardens or large containers. It has been assessed as suitable for potential floriculture and landscape uses, and flowering stems are excellent for decoration.



# Seed characteristics

Each seed in an isopogon or petrophile cone comes packaged within a hard, dry fruit. They are simple indehiscent dry fruits meaning the fruits are dry when mature, are formed from one ovary, and do not break open when ripe. Within each cone are many fruits, making the cone an aggregate fruiting head (or infructescence).

The base of each fruit is often broader than the tip, or apex. Dried remains of the base of the floral style often remain attached at the tip like some sort of tail. The fruits of both genera have external additions in the form of hairs or a narrow wing, which change the shape and look – isopogon fruits are a bit like a shuttlecock, and petrophiles tend to look like a hairy tick or small beetle, or sometimes more like a stingray in shape.

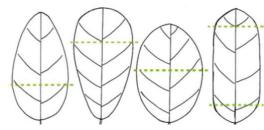
**Isopogon fruit** are rounded, ovoid (egg-shaped) to elliptical. Fruits are covered all over with fine, white, spreading hairs, longer at the base and becoming progressively shorter towards the apex. The longest hairs are longer than the fruit itself. In fact, the fruit is so hairy the hairs tend to obscure the brown/black fruit underneath. The fruit are attached at the base to a woody core or shallow receptacle, and radiate out at a wide angle in



between cone scales also attached at the base to the same core, and other packing material (or chaff). Isopogon fruits are taller than wide, generally around 3-4 mm long with a few exceptions such as *I. inconspicuus* (2 mm) or *I. autumnalis* (5-5.5 mm long). Pictured left, elliptical shape. Far left, ovoid shape. Above right, an *Isopogon dawsonii* fruit.



**Petrophile fruit** are more-or-less flattened so are essentially two-dimensional, the front and back shallowly and unequally convex on each side. The brown fruits are generally



slightly taller than wide and the shape in outline is usually ovate or obovate, sometimes elliptical or oblong.

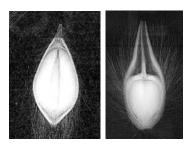
From left: ovate, obovate, elliptical, oblong shapes. Right, *Petrophile ericifolia*. Below left, *P. serruriae* has a tuft or coma.





Many petrophile fruits have a beak at the tip formed by the base of the style and some may have an indented base. Most have long hairs on the margins and/or (pictured left) a long tuft of hair at the base. These hairs vary in colour from white to brown, even reddish-brown or fawn. The sides may also have shorter felty hairs on part or all of the two flatter surfaces. Some species have a narrow, papery, flat wing surrounding the seed instead of hairs. The fruit are attached at the base to a woody core and lie roughly upright or parallel to the core beneath cone scales also attached at the base to the same core. Petrophile fruits vary in size from as small as 2 mm long up to 8-10 mm long (often including an apical beak).

Seeds of isopogons and petrophiles are hidden inside a fruit and are hard to see. Under x-ray, you can see there is something inside the fruit. Seeds are rarely extracted from the fruits because it is difficult to do so without damaging the seed. We would expect seeds to be only slightly smaller than the fruits. In isopogons a few measurements are available which indicate that the overall length of the seed may be around 1 mm or less than the overall length of the fruit.



Right: X-ray fruit images (Dave Bishop), left, Isopogon anemonifolius; far right, Petrophile circinata

# Nature's seed bank: on-plant (canopy) storage

The seed storage strategy employed in isopogons and petrophiles is retention in woody cones in the canopy. This seed bank releases its seed in response to an ecological trigger such as fire, rather than spontaneously at seed maturation where seed is then stored in the soil (the more common seed storage strategy). About 20-30% of the southwest WA sandplain flora exhibits canopy storage, or serotiny, and it is considered extremely common among the proteaceae of this area. The incidence of canopy storage increases with decreasing average annual rainfall in this area. The term bradyspory is also used in relation to canopy seed storage, referring to the release of seed or fruit from a cone over time as opposed to the release of seed as soon as they have matured.

Serotiny in isopogons and petrophiles tends to be taken as a given in the scientific literature. While all the species can store seed in cones, the serotinous traits of individual species are rarely explored. These genera are either broadly categorised as serotinous or labelled as genera containing serotinous species. There is no clear evidence that any isopogon or petrophile species is non-serotinous, unlike banksias for example, where Banksia integrifolia is one of only a few non-serotinous species.

The level of serotiny varies across genera and across species. In isopogons and petrophiles, species are considered to be serotinous but at low/weak or moderate levels – meaning that most seed is released within a year of maturity (weakly serotinous) or released within five years of seed maturity (moderately serotinous). Plants may also release some of their seed spontaneously in the absence of a trigger (weakly serotinous). No species are

| Snecies | named | ns s | erotinous | in | scienti | fic litera | ture |
|---------|-------|------|-----------|----|---------|------------|------|
| Species | numeu | us s | erounous  |    | SCIEILU | nc niciu   | luie |

| species named as sciotinous in scientific iterature |                 |                                  |  |  |
|---|-----------------|----------------------------------|--|--|
| Weak serotiny                                       | Medium serotiny | Serotinous (level not specified) |  |  |
| I. dubius   | P. anceps       | I. anemonifolius                 |  |  |
| I. divergence                                       | P. drummondii   | I. anethifolius                  |  |  |
| I. sp.  | P. filifolia    | I. dawsonii                      |  |  |
| (unknown species                                    |                 | I. dubius                        |  |  |
| from the Stirling                                   |                 | I. formosus                      |  |  |
| Range, probably I.                                  |                 | I. petiolaris                    |  |  |
| spathulatus)  |                 | P. pulchella                     |  |  |
|   |                 | P. sessilis                      |  |  |

known to be strongly serotinous, retaining seed for extended periods of time e.g. 5-15 years or longer, or even indefinitely. In one study (Yearsley, Fowler and Ye, 2018), the petrophile species studied had higher levels of serotiny (moderate) than the isopogon species (low serotiny).

Serotiny levels vary according to climatic conditions and the local fire regime. In WA, weak serotiny is associated with more coastal and wetter southern parts of the southwest, beside swamps and on rock outcrops, and also areas where crown-fire is infrequent. These are also areas where many isopogon and petrophile species occur. The eastern species also come from coastal and/or wetter areas, a possible exception being *Isopogon petiolaris*, where many populations grow away from the coast in dry sclerophyll forest and heath, commonly in stony sites, on the tablelands and west to the plains. Serotiny levels can even vary among populations of the same plant species depending on the site characteristics. For example, in the east, *Banksia serrata* populations range from complete dependence on fire for population maintenance to bet-hedging (spreading potential recruitment between fire events and inter-fire periods).

### Seedbank dynamics

Progressive deposits of new fruits and seeds are required to grow the seedbank held on plants. New deposits are not ready until the new fruits have matured and are germinable. In practice, isopogon and petrophile cones are considered to have matured by six months after flowering (indicated by a grey, aged appearance). In unburnt areas, isopogon and petrophile seed dispersal is often observed in autumn/winter, 6-9 months after spring flowering. The scientific literature records that *P. sessilis* fruit takes 8 months or more and P. pulchella takes up to a year.

Petrophile pulchella and Petrophile sessilis are both reseeders. Deposits to the seedbank of new fruits and seeds are greater in reseeders than in resprouters, as reseeders tend to produce greater numbers of seeds per plant making their annual reproductive output generally higher than in resprouting species. Petrophile pulchella could potentially accumulate a maximum seed bank of ~1500 seeds per plant (Jenkins, Morrison and Auld, 2010). The seed store is more critical to the survival of reseeders after fire than resprouters because they are killed by fire. Adults die but regenerate from many small seeds.

Deposits of new fruits and seeds are balanced by withdrawals (losses) from the seedbank, which can have a major impact. In one study of *P. sessilis*, the germinable seed bank remained almost constant over a 12-month period due to seed predation or spontaneous seed abortion, with new seeds making an increasing contribution. In *P. pulchella*, the main cause of seed bank losses is reported as spontaneous fruit rupture (nearly three-quarters of the oldest *P. pulchella* bracts had ruptured), and therefore seed release and loss. This was related to cone age, with up to 25% of older *P. pulchella* fruits open.

#### Low seed set/low seed fill

Sometimes seed is not formed (set) at all. This is common among proteaceous plants. One estimate of seed set across 24 species in nine genera of Australian proteaceae was as low as 3.4%. Factors causing low seed set include pollination failure, limited resources, environmental stress, genetic constraints or predation. Studies of proteaceae species have shown that low nutrient levels, or limited resources, leads to fewer seeds. Studies on banksias indicate that inflorescences appear to be able to fine-tune annual reproduction in line with fluctuations in resource availability and/or variations in pollination success. When resources are limiting (low nutrient availability), plants may produce fewer seeds although seed size is not reduced as well in species with small seeds (such as isopogons and petrophiles).

In other cases, the seed is formed but is not filled by a viable embryo. Seed fill is a measure of the proportion of outwardly undamaged fruits that have seeds with the tissues essential for germination (that is, an intact embryo). In Australian plants, it is common for a high proportion of seed in each annual cohort to be aborted.

Cones and fruits of isopogons and petrophiles usually provide no outward indication of whether there are any seeds within, and if those seeds are likely to be viable. This is very different to other proteaceae such as banksia or hakeas where swollen follicles are a clear indication that a seed has formed inside which is likely to be viable. According to the Western Australian Threatened Flora Seed Centre, seed fill (presence of intact seed) in isopogons and petrophiles is less than 10%. Therefore, most of the fruits in each isopogon or petrophile cone are likely to be empty; fruits are formed but



Isopogon anethifolius: plump viable fruit, outnumbered 3 to 1 by apparently non-viable ones. Photo John Knight

not filled by a seed. These false fruits are the result of aborted seed or lack of proper fertilisation. Some false



Isopogon anethifolius: 396 woody bracts! Photo John Knight

fruit can be identified by observation (small size or weight or some deformity), but others look undamaged.

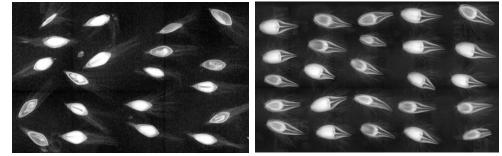
From three cones of *Isopogon anethifolius*, SG member John Knight counted 396 floral bracts (cone scales) from three large cones (pictured right), and 214 fruit. If the cone scales equate to 396 flowers, seed set was 54% but could be

higher given that isopogons often have bare floral bracts at the top of the flower head. At this stage, it is not clear how much seed set varies across species. Note that *I. anethifolius* is a reseeder, which tend to have higher seed set than resprouters.

Of the 214 *Isopogon anethifolius* fruits, John assessed 63 as fertile (pictured right). This indicates seed fill of 29.4% which is relatively good compared to the 10% we might expect. However, even the healthy-looking fruit may be false fruit. The only way to confirm the presence of seed is to cut the fruit open. When SG member Tony Henderson tried this with *Petrophile circinata*, he found fruit that looked perfect had nothing inside; the whole batch of fruit was empty! This species was also the subject of an x-ray taken by SG member Dave Bishop (below right) which shows that of the 25 fruits in the frame, 14 seem to have less substance than the others although they are of a similar size. Dave also took an x-ray of *I. anemonifolius* (below left) which shows that about ten of the 24 fruits do not look intact.

#### Loss of germinability

A greater level of germinability in fresh seed versus old seed has long been reported in the study group. Ralph (2011) also reports that viability in petrophile seeds appears to progressively decline over several years. There is also



evidence for this from a study which found that in *P. sessilis* seeds in older fruits rapidly lost germinability (Brown and Whelan 1999). Over the course of a year, germinability of two-year-old fruit fell by 15% early on, followed two months later by another 15% fall (a total reduction of almost 30%). This rapid loss in germinability was offset as new fruits matured to replace them, thus replacing the seed store annually. In comparison, in this study the co-occurring *Hakea sericea* seed remained viable for longer than *P. sessilis*.

Not all species lose viability in seeds as quickly as *Petrophile sessilis*. When harvested and kept in storage, some seeds can remain germinable for many years. SG member Royce Raleigh managed to germinate 14-15 year-old seed of a species of petrophile. Staff and volunteers at the Eurobodalla Regional Botanic Gardens were able to germinate seed of I. *anethifolius* stored for 24 years (although only 9% germinated). Storage conditions are very important. Isopogon seed stored at the Western Australian Threatened Flora Seed Centre in tightly controlled laboratory conditions showed no decline in viability after ten years. Even when dropped from cones to the soil below the host plant, fruits of *I. anemonifolius* and *P. pulchella* have been found to remain germinable for nearly two years (seedlings emerged up to 700 days after fire).

#### Predation

Serotinous seeds are more nutrient-enriched, so good protection over time in woody cones is vital to prevent high levels of pre-dispersal predation. In western species of isopogon and petrophile, pre-dispersal protection is rated as moderate (Groom and Lamont 2015). In comparison, other (more serotinous) western genera such as banksia and hakea offer strong protection. In the eastern species *P. sessilis*, the protection offered by softer cones with many points of weakness at the numerous cone scales is much lower than the protection provided by other species which have thick, woody fruit such as *Hakea sericea*. A comparison of the two species showed seed predation within retained cones was much greater in *P. sessilis*, and in older cones was substantial. *Petrophile sessilis* suffered a greater percentage loss of cones each month than *H. sericea* and progressive loss in older fruit was seen only for P. sessilis (Brown & Whelan 1999).

The main predators of seed stored in woody cones or fruit are boring insect larvae and strong-billed birds. In proteaceous species, infructescences (cones) are highly predated by lepidopteran larvae (butterflies and moths) and studies show petrophile species are sensitive to such seed predation. Insect predation of the seedbank has been estimated at 20-30% for *Petrophile pulchella* and some eastern banksias. Older petrophile cones have much higher levels of seed predation compared to younger cones. Newly produced cones/fruits show no signs of destruction because the larvae would not have developed sufficiently to tunnel through the fruit and into the seeds. In *P. pulchella*, much higher levels of predation (over 50%) in old cones (over five years old) are reported compared with young (around 5% in one year old) cones. Similarly, Brown and Whelan (1999) found higher levels of insect predation in *P. sessilis* (up to around 75% of seeds destroyed) in older cones (over two years old) compared with younger (1-2 years old) cones (less than 20%).



Insect larvae damage. From left: young and older isopogon cones, petrophile cone with frass, petrophile fruit.

Birds such as cockatoos and other parrots feed on the larvae inside fruit, on green seed pods and on seed of many proteaceous species. Black cockatoos specialize in extracting seeds from woody fruits. In petrophiles, cockatoos are seed predators but the main predators are insect larvae. In *Petrophile sessilis*, insect damage (individual seeds) is more common than bird damage (removal of the entire cone). Petrophiles allow birds easy access to the seeds and/or insect larvae tunnelling inside. In *P. sessilis*, fruit removal did not vary across months, suggesting that birds are attracted to this food source throughout the year (Brown and Whelan 1999).



Left, female Gang-gang (photo Alan & Dianne Page); right, male Gang-gang (still courtesy Tessa Barratt); bottom right, P. *pulchella* with entire cone ripped off. Location: Blue Mountains NSW.

Black cockatoos remove entire cones of both western and eastern species of isopogons and petrophiles, holding them in one claw (reportedly the left) while chewing. In WA, *Isopogon scabriusculus* is used for feeding by the endangered Carnaby's black cockatoo and is considered medium priority for planting for

this purpose. *Isopogon uncinatus* is documented as important for Carnaby's Cockatoo as well as the threatened Baudin's Cockatoo. In eastern Australia, endangered Ganggang Cockatoos have been observed really getting into cones of *Petrophile pulchella*. There are three sightings recorded by citizen scientists in the Blue Mountains/greater Sydney area involving a female, male and a flock respectively. As one of our smallest cockatoos (only the Cockatiel is smaller), Gang-gangs prefer softer, more accessible



woody cones like petrophiles over larger ones like banksias. The flock of Gang-gangs was actively engaged in

chewing young cones and were not attending any other plants growing in the area. The female Gang-gang was also gnawing young cones while the male Gang-gang was chewing mature cones on a dead plant.

## Seed release triggers

In serotinous species, seeds are kept stored in tightly closed cones in the canopy until the cones are triggered to open by an environmental cue. The many possible triggers include:

- Death/damage of the parent plant or branch (necriscence)
- Age (senescence)
- Wetting (hygriscence)
- Warming by the sun (soliscence)/exposure to sunlight
- Drying atmospheric conditions (xyriscence)
- Fire (pyriscence)
- Fire followed by wetting (pyrohydriscence)

The most studied trigger is fire, heat from flames causing seed release. The term *serotiny* is frequently used as shorthand for *pyriscence* referring to fire as the trigger. This fire-mediated serotiny is an adaptation to fire-prone environments. Canopy storage maximises the quantity of seeds available for the next post-fire generation. (The fire may also initiate other release triggers such as death/damage to the plant and drying or heat.)

Woody cones typical of serotinous species provide thermal insulation from fire-induced heat. However, cones are subjected to fire-induced heat over seconds, not minutes, and are not designed for absolute heat protection. In isopogons and petrophiles, the small one-seeded fruits are held between or under cone scales and are tightly clustered in oval or globular heads which helps protect individual fruits. As cone scales are generally thinner in isopogons than in petrophiles, isopogon fruits are also cocooned in hairy or fluffy packing material (chaff).

Young isopogon cone scales are often woolly or hairy, the hairs likely providing additional protection from fire. Sometimes the involucral bracts also have similar furry coverings for further protection. Petrophiles, on the other hand, may also have such hairy protection on cone scales and involucral bracts, but the trait is not consistent across species (petrophiles being already well protected by thicker, woodier cone scales). The thicker cone scales of petrophiles may reflect a greater degree of serotiny compared to isopogons.

With age, cone scales harden and become woody. The most important heat protective features in woody cones are the wood density, moisture content and wall thickness. Generally, the greater the level of serotiny, the greater the mass of the supporting fruit/cone. Compare the mass of banksia cones (many of which are strongly serotinous) with the smaller size, softer mass and thinner outer of isopogon or petrophile cones.



In banksias, fire melts resin sealing the seed follicles shut and to some extent it is possible that the same mechanism operates to allow cone scales in petrophiles to open. Bees have been observed collecting resins from sticky plant parts of petrophile flower heads at the immature, flowering and spent stages. The sap-like resin is used to make propolis to seal gaps in the hive. Exuded from flower and leaf buds, it forms a defensive coating, protecting plant parts from pathogens, fungi, and insects. Viscid floral structures (involucral bracts and/or tepals during flowering, and cone scales or the whole flower head before and after flowering) are common amongst short-leaved western petrophiles, such as *P. ericifolia*, *P. merrallii* and *P. cyathiforma*, and particularly in the *Petrophile scabriuscula* complex (*P. scabriuscula*, *foremanii, recurva, globifera*). *Petrophile drummondii* and *P. biternata* also have viscid involucral bracts (although with longer and divided leaves).

Left: bees collecting resin from petrophiles. Top, collecting resin from an immature, sticky flower head. Bottom, *Exoneura* sp. collecting beads of resin from a spent flower head. (Photo: Jean & Fred Hort).

#### Other release triggers

Some plants respond to more than one trigger to release seed from canopy stores. As well as fire, death of the parent plant or branch triggers release of seed from the canopy store. Based on bush observations over 25 years, SG member John Knight notes the germination of isopogons and petrophiles in southeast Australia killed by tree clearing, logging, or grading along roadsides can be almost as generous as mass germination following fire. Warmth from the sun and dry conditions could also be triggers, or cycles of wetting and drying. In the Sydney region, researchers consider alternate wetting and drying to be another seed release trigger in *Petrophile pulchella* and *P. sessilis*, but not in *Isopogon anethifolius*.

Releasing seed in the absence of fire is considered a 'bet-hedging' strategy in weakly serotinous species, allowing limited inter-fire recruitment in habitats that experience long periods without fire. This release of seed can be readily observed in the bush. Species photographed 'in the act' by the study group include live plants of *I. anemonifolius*, *I. fletcheri*, *I. longifolius*, *I. linearis*, *I. ceratophyllus*, *P. longifolia*, *P. fastigiata*, *P. serruriae*, *P. megalostegia*, *P. pilostyla*, and *P. teretifolia*.

Seed release in the absence of fire has also been observed in cultivation. Members have reported *I. dubius*, *I. anethifolius*, *P. sessilis* and *P. pedunculata* spreading spontaneously in their gardens. SG members Mark and Carolyn Noake found *I. anethifolius* particularly successful at establishing itself in the open ground of their garden path where competition from other plants was minimal. In sandy, dry conditions in the Special Collections at Cranbourne Botanic Gardens near Melbourne, the isopogons and petrophiles thrived so well that thirty years later and in the absence of fire, their offspring now dominate the plantings. *Petrophile sessilis* and *P. pulchella* have actually shown weedy tendencies, reproducing so vigorously the fence was opened to allow animals help control plant numbers. Five western species are now considered locally common there (*P. squamata*, *I. baxteri*, *P. diversifolia*, *P. glauca* and *P. cyathiforma*).

While fire is not essential for seed dispersal, post-fire environments have significant advantages. Fire-mediated serotiny or pyriscence is an adaptation to a fire-prone environment which maximises seed availability for release post-fire, ensuring that the simultaneous release of the seeds coincides with the presence of favourable conditions for germination and seedling recruitment. Fire reduces competition by clearing out undergrowth, maximising water, nutrient and light availability to seedlings. The resulting bed of ash raises the pH of the soil and temporarily increases soil nutrition. The impact of predation of seeds on the ground is also reduced by the release of a large number of seeds at once, and the temporary satiation or elimination of predators.

In unburnt vegetation, there is a general absence of seedlings and very young plants – short-term mortality of seedlings is high. Most serotinous species tend to rely mostly on fire for seedling recruitment although new cohorts can sometimes be found in long unburnt stands of bush. *Petrophile pulchella* is one of the species using the inter-fire period for recruitment, presumably reflecting a larger number of seeds released, but the likelihood of plant recruitment and survival to reproductive age from the seeds released in the absence of a fire is considered extremely small. Another species using inter-fire recruitment is *Isopogon crithmifolius*, numerous young plants of reported by SG member Lyn Alcock in the Dryandra Woodland National Park in WA where there has been no fire for many years.

Key reasons for this inter-fire seedling mortality are predation of seed and too much competition. In a comparison of young seedlings of *I. anemonifolius* and *P. pulchella* in control plots in unburnt and burnt areas, there was almost total destruction in unburnt areas (likely due to rodents) compared to no losses in the burnt area (in Myerscough et. al.). However, some species (those with weak serotiny) may prefer litter-filled microsites over bare areas, for example, *I. anemonifolius*. Although inter-fire recruitment is less successful in the short-term than post-fire recruitment, over long periods (up to 20 years) inter-fire recruitment catches up and net recruitment is ultimately quite similar.

### Seed dispersal

The dispersal unit in isopogon and petrophile cones is the diaspore consisting of a seed inside a fruit. Isopogon and petrophile fruits are adapted for dispersion by wind (anemochorous).

#### Emergence

When the cone begins to break up (isopogons) or the cone scales open (petrophiles), pressure holding the fruit in place is released. Long hairs on the fruit, kept tightly bound against fruit before dispersal, spring open. In *Isopogon latifolius*, SG member Liesbeth Uijtewaal discovered another way the hairs are kept firmly closed: the remains of the floral tube enclose the hair ends much like a band on a ponytail. When this falls away, the 'parachute' of hairs can open (before and after, pictured right).



The longest hairs are located at the base of the fruit deep inside the cone. With the pressure relaxed, the hairs spread to push the fruit out and away from its surrounds. The fatter the seed/fruit, the more the hairs push out. This mechanism is most pronounced in isopogon fruits, the hairs fluffing out three-dimensionally to 'pop' out from their packing material. Liesbeth describes the way the hairs work the fruits out of the cone as like swimmers' arms working their way to the surface after a dive.

#### **Delayed dispersal**

Once freed from protective structures (chaff in isopogons, thick cone scales in petrophiles), fruits initially remain with the cone structure. This is facilitated by their extremely light weight and by the way the hairs interlock with those on other fruit and with cone structures to form a loose, lightweight bundle lightly attached to the remnants of the cone. It is readily observable in the bush and in gardens when cones start to look fluffy.



From left: *P. megalostegia*, *P. crispata*, *P. fastigiata* (photo Liesbeth Uijtewaal), *I. anemonifolius* (photo Karlo Taliana).

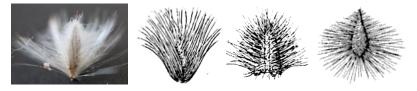
Isopogons and petrophiles are serotinous as well as wind-dispersed, both attributes which are associated with a pause between seed release from cones and seed dispersal. However, the interval length of the pause is related to the level of serotiny so the pause in isopogons and petrophiles is not as long as in species with stronger serotiny.

#### **Primary dispersal**

The initial dispersal is a few metres on to the ground. Wind-dispersed seeds come in two main forms: winged and plumed. Isopogon and petrophile fruits are almost all plumed (hairy). Hairs aid wind dispersal, catching the wind so that the descent speed slows. Weightless structures like hairs or wings enhance wind resistance, provide buoyancy and increased volume, enabling the slightest breeze to pick them up. Unlike other species, fruit are generally only about a metre high on average, so they cannot depend on height to help seed travel.

**Isopogon**: The silky hairs all over isopogon fruit graduate in length from longer nearer the base, to shorter at the tip. They radiate out from the fruit at a wide angle. The resulting three-dimensional shape is like a shuttlecock. These hairs and shape make the overall volume of the disapore greater in isopogons than in petrophiles.

Fruit hairs act as an umbrella or parachute which turns the fruit upside-down and slows its descent. Trials show they descend slowly, drifting and landing either point down (short hairs) or on their side. Sideways travel occurs even with minimal air movement. Across species, isopogon fruits are generally similar in design. Although shaped like a shuttlecock, these fruits are not weighted at the base like a shuttlecock (designed to travel very fast, and always land base down) and move quite differently through space.



A selection of isopogon fruits from different species.

**Petrophile**: On flattened petrophile fruit, there are a range of structures to aid wind dispersal. Most have long hairs on the side margins. In some species, there is a dense tuft of long hair (coma) at the base which lies to one side of the seed. The coma can also extend up the margins.



A selection of petrophile fruits: *P. imbricata*, *P. linearis*, *P. wonganensis* and *P. axillaris* (with coma). Not to scale.

Some petrophile species use narrow, papery

extensions of the fruit as 'wings' to catch the wind. This allows them to glide as well as drift. These narrowly winged petrophile fruits are lighter and do not need as much wind to travel as larger banksia or hakea seeds with larger wings. There are nine petrophile species with winged fruits, with a variety of designs although most resemble a stingray in outline.





From left: *P. diversifolia*, *P. heterophylla*, *P. glauca*, *P. carduacea*. Not to scale.

winged fruits biloba carduacea chrysantha divaricata diversifolia glauca heterophylla plumosa squamata

These hairy or winged structures around fruits slow descent. Petrophile fruits drift or glide on air currents. Trials showed the descent was faster than in isopogons and, without discernible air movement, they did not drift. They land on one of the two sides.

#### Secondary dispersal

Fruits may later be carried by wind or water across the ground until they are permanently trapped by leaf litter, fallen twigs or other obstacles, or in soil depressions. The duration of wind-assisted seed movement is dependent on when a seed becomes entrapped by surface obstacles or covered by soil, or when the first heavy rains occur. Secondary dispersal can cover several kilometres. Animals may also assist dispersal. *Isopogon trilobus* fruit has been found in emu droppings. Although ant dispersal of seed is common (particularly in southwest WA), it has not been recorded in isopogons and petrophiles.

On dispersal, the fruit does not break open to release its seed. Only germination splits the fruit. However, not all seeds germinate immediately on dispersal. The hard fruit wall covering seeds of isopogons and petrophiles is unlike other serotinous species which generally have soft seedcoats and germinate immediately. Seeds that have been lying on the ground for many months still germinate readily when conditions are appropriate. In studies of *Petrophile sessilis*, new seedlings appeared as much as 15 months after fire. In some sites, the first seedlings did not appear for over six months after fire. Similarly, after the Black Summer fires of 2019-20, some sites had no isopogon or petrophile seedlings 9-10 months afterwards. In *I. anemonifolius* and *P. pulchella*, seedlings can emerge up to 700 days after fire. SG members and propagators Tony Cavanagh and Keith Alcock have reported delayed germination in relation to 12 western species of seven and 9-10 months respectively after sowing seed, with germination eventually occurring in cold autumn/winter weather. Having previously tried sowing in cold weather without success, Keith suggests that time in/on the ground could be an important factor.

However, as discussed, seeds on the ground are highly vulnerable to predation. In unburnt areas, rodents appear to account for very high levels of predation. A small seed size may help isopogon and petrophile species avoid bird predators, unlike plants with large seeds. Some researchers have classed isopogon and petrophile fruit as cryptic, meaning they blend in with their background and are hard for predators to detect on certain soil types (Pausas and Lamont 2018).

### Germination

Species

Isopogon linearis

Isopogon tridens

Petrophile biloba

Petrophile linearis

Petrophile media

Petrophile striata

Petrophile chrysantha

Petrophile drummondii

Petrophile macrostachya

Isopogon sphaerocephalus

Within each fruit, a seed contains a very small embryo (an immature plant with a store of nutrients to help it grow). It has two main parts – a radicle that will develop into a primary root, and a cotyledon, a primary leaf. The fruit is hard-coated but permeable, allowing humidity to get in to enable germination of the seed. As the embryo begins to

grow, the radicle or root tip expands beyond the fruit then the cotyledons begin to emerge, finally splitting the fruit.

Right, rootlet emerging from the base of a petrophile seed (photo: Liesbeth Uijtewaal). Middle, petrophile cotyledon with empty fruit still attached. Far right, seedling with cotyledons and first leaves (Liesbeth Uijtewaal).



Germination rates in isopogons and petrophiles have not been widely or satisfactorily measured. Hindering the assessment of germination results is different inputs to calculations, and lack of information about these inputs. In most cases, all fruits collected are sowed while in others, only fruits which pass certain viability tests are sowed. In addition, tests of seed viability are highly variable or non-existent. This means the denominator used in rate calculations varies wildly, so germination rates are likely to be grossly underestimated according to the level of seed fill.

High germination rates in isopogon and petrophiles are reported when only viable seed is used (see table, right). An

Perhaps the low

germination

experiment by Yearsley, Fowler and Ye (2018) tested first for viable seed using a cut test, and calculated germination rates in western species ranging from around 55% to around 95%. In the eastern species P. sessilis, Brown and Whelan (1999) found the proportion of intact seeds that germinated varied by age, peaking at around 70% at around two years after flowering.

unknown)

18%

1%

13%

30%

58%

24%

0%

2%

60%

0%

Germination rate (seed viability

| Species                   | Germination rate |
|---------------------------|------------------|
|                           | (viable seed)    |
| Isopogon divergens        | Around 55%       |
| Isopogon dubius           | Nearly 90%       |
| Isopogon sp. (probably I. | Nearly 90%       |
| spathulatus)              |                  |
| Petrophile anceps         | Around 60%       |
| Petrophile drummondii     | Around 95%       |
| Petrophile filifolia      | Nearly 60%       |
| Petrophile sessilis       | Up to 70%        |

Germination rates averaged across experimental groups.

rates generally reported by study group members are underestimates due to the presence of non-viable seed sowed alongside viable seed. Other sources report similarly low germination rates but also great variability in germination rates across species. As there is no information available on whether the fruits used were checked for intact seed and other viability tests, the variability may also reflect the presence of non-viable seed. Bell, Plummer and Taylor (1993) collated results from three sources on selected western isopogon and petrophile species (listed left). However, the large variation in

rates suggests that non-viable seed may have been present alongside the viable seed. Compare the results for P. drummondii (24% versus 95%).

Other published germination results include Ralph (2011) who assessed Petrophile drummondii germination as moderately low – a rate which has more in common with Bell et. al. as listed above than the Yearsley et. al. result using intact seed. Ralph also reported P. linearis viability as poor. His results were generally low, recording a maximum of 35% for I. ceratophyllus and poor to moderate with germination staggered over a long period for P. canescens, P. macrostachya, P. media and P. striata. Only in P. conifera did fresh seed appear to germinate readily. He concluded that viability of petrophile seed varies between species.

In the study group there has been considerable debate over which factors might trigger the germination of isopogon and petrophile seed. Study group trials have shown that most species will germinate readily from fresh seed without pre-treatment. A wide range of pre-germination treatments such as smoke-water, pre-soaking, even singeing hairs or slitting seed have not demonstrated any clear benefits. In 2005, SG member Mic Forster compared the germination rates of *P. pulchella* fruits which were pre-soaked in water for 48 hours with those left untreated. He found a statistically significant difference between the germination results of the two experimental groups – germination (defined as the development of the cotyledons) was higher in the group with no pre-treatment (48%) than the group with pre-treatment (19%). Another experiment found little effect from heating *P. sessilis* cones/fruits at 400° C on the percentage of intact seeds that were germinable (Brown and Whelan 1999). Historically there has been speculation that the hairs on fruit of isopogons and petrophiles might be hydrophobic and thus repel water from the seed. Study group trials have shown, however, that removing the hair is not necessary and that there is no evidence to support this theory.

According to the scientific literature, the key trigger for germination in serotinous species like isopogons and petrophiles is rainfall, based on observations of the timing of germination in relation to seed release and rain events. In WA, winter rain has been demonstrated to be more important for germination than smoke, wall decay or light.

*Isopogon dubius* will germinate with only a few drops of rainwater pooled in the flattened bottom of the fruiting cone, as SG member David Lightfoot found in the WA kwongan. There had been good rain in the area over winter and, spotting a hint of green in a fruiting cone in late winter, he found that two seeds had germinated in situ. After planting out in pots the germinants shot away, happy to get their roots into potting mix rather than the air.

Many study group members advise that fruits must be kept well-watered to germinate seed in their nurseries. The importance of water in germination was demonstrated with great success in a trial of *Petrophile pedunculata* by SG member Mark Noake with what he called the "bog" method. To keep the fruit moist, the punnet was placed into a slightly larger container holding water with drainage holes about 1 cm up each side.

In western species, the germination stimulus is, more precisely, winter rain (in Groom and Lamont, 2015). In WA, rainfall is strongly seasonal, so germination reliably occurs in winter. In the Sydney region, there is not strong seasonality of rainfall and so the timing of the first opportunity for germination after fire is quite unpredictable.

Temperature is also important for germination. For many Australian species in temperate and Mediterranean climates germination tends to be best at temperatures associated with the winter rainfall period (approx. 15-20° C), according to Sweedman and Merritt (eds, 2006)). The coincidence of rain and lower temperatures in winter in Western Australia creates perfect conditions for germination. In eastern Australia, ambient temperature is reported to have a strong effect on germination of *Isopogon anemonifolius* and *Petrophile pulchella*. Diurnal cycles of high temperatures typical of summer conditions imposed secondary dormancy on seeds irrespective of moisture availability, whereas germination levels were maximal under cool conditions typical of winter when moisture was nonlimiting. Reseeders tend to have narrower optimum germination temperature ranges than resprouter species. This is one of the few seed trait differences between reseeder and resprouter species; there is no basic difference in mean seed mass, viability (ability to survive or live successfully), or germinability of seeds.

Evidence on the effects of temperature on seed germination is also available from some leading isopogon and petrophile propagators. In one trial of seven species, Ralph (2011) found results were substantially higher when seed was germinated at a constant 15°C when compared to seed that was sown in a glasshouse. He suggests that in southern Australia, seed is best sowed in autumn. Legendary plantsman Ken Stuckey had great success in the 1960s with a huge range of isopogon and petrophile species sowed in late March (early autumn) when temperatures in South Australia were beginning to drop. The Sutherland Shire Community Nursery in Sydney, which propagates large numbers of eastern isopogon and petrophile plants every year, has found that cooler months with daytime temperatures less than 24° C, are best for germination. At the Eurobodalla Regional Botanic Gardens, the pattern of germination in *I. anethifolius* and *P. pedunculata* shows winter sowing is more successful. They suggest aiming for when there is the largest difference in day and night temperatures. In a trial, SG member Tony Cavanagh found that of ten western isopogon species sowed in October, all germinated except one (*I. latifolius*) which did not germinate until seven months later when cold weather arrived. He replicated this result by sowing more seed from the same batch in cold weather in May, resulting in germination of over 70%. This indicates that while some species will tolerate a range of temperatures for germination, *I. latifolius* will not. In another cold climate species, *P. canescens*,

Ralph (2011) found that a short period of chilling seed (5°C for a week) improved germination results (but only up to 40%).

There are always some species that do not follow the rules and one of them is the western species *Petrophile heterophylla*. In a study of the effect of different sowing times (mid dry season i.e. January, late dry season i.e. April, and at the break of season i.e. May), *P. heterophylla* showed the highest seedling emergence from sowing in January and the lowest from sowing in May (Worthington et al., 2006). This early sowing may secure a competitive advantage through germinating early in the season, but risks higher mortality in the event of sporadic rainfall and exposes fruit on the soil surface to desiccation, predation and dispersal for many months.

In native plant communities of southwestern Western Australia, an after-ripening period of warm, dry storage is reported to help increase percentages of germinable seeds. The Sutherland Shire Community Nursery has found that storing before cleaning and sowing helps some species have an improved germination rate. For example, *Isopogon* and *Petrophile* seed that has been stored for six months before being cleaned and sorted have a much better result than if they were to be cleaned and sown straight away. As discussed above, not all isopogon and petrophile seeds germinate straight away on the ground in nature, indicating to some extent, adaptation to delayed germination. This may also apply to *P. heterophylla* discussed above.

#### Smoke

Most assume that Australian species require smoke for seed to germinate, but the seeds of serotinous species rarely require any further cue for germination once seeds are released from the woody fruits. Smoke or heat from fire triggers cones to open but the fruits inside do not tend to emerge straight away into the smoke-filled environment. In contrast, species which store their seed in the soil require triggers such as smoke, fire or heat to germinate.

There is evidence for this from scientific studies. Yearsley, Fowler and Ye (2018) tested the effect of smokewater on germination in eight western serotinous species including *Isopogon divergens*, *I. dubius*, *Petrophile anceps*, *P. drummondii*, *P. filifolia*, plus another unidentified isopogon from the Stirling Ranges (probably I. *spathulatus*). They found no germination benefits from smoke water treatment. Pausas and Lamont (2018) report that isopogons and petrophiles are not stimulated to germinate by fire (or rarely). In *Petrophile pulchella*, Myerscough et. al. (2001) report no difference in levels of germination between fruits taken from cones burnt in a fire of moderately high intensity and in those from unburnt cones. Study group findings over many years are consistent with this, with no clear benefits demonstrated using smoke for germination.

Turning to Australian seed guides, Sweedman and Merritt (eds.) 2006 list isopogons and petrophiles as reportedly having smoke-responsive seeds. A study of ten different pre-treatments (including hormone, heating, light, removing bracts and nicking seed) in the same guide included 20 isopogon species and 24 petrophile species (all western) but the only pre-treatment recommended was smokewater for petrophiles with no pre-treatment recommended for isopogons. In another seed guide, Ralph (2011) reported that smoke treatment enhanced results for the eastern species *I. ceratophyllus* from nil to 35%. He found that smoking seed prior to sowing provided better results than smoking seed sown into trays. In petrophiles, his results were variable. For western species *P. drummondii* and *P. linearis*, smoke treatment significantly improved results and quickened germination. For eastern species *P. canescens*, germination was improved with smoke treatment, but results were still less than 20%.

Large-scale propagators apply smoke indiscriminately to seeds of all Australian plant species, so do not test whether smoke improves germination. (The largest producer in Australia, Native Plant Wholesalers, uses cuttings in preference to seeds to propagate isopogons.) One such propagator, Sutherland Shire Community Nursery, often applies smoke to isopogon and petrophile fruits using a bee smoker with eucalyptus leaves rather than smoke water, but are not convinced this is important. The smoke itself could be a factor, as aerosol rather than liquid smoke has been documented to stimulate different effects to smoke water.

# Seedling fitness/vigour

Germination success is pointless if the resulting seedlings do not thrive and survive. Seedlings are established when cotyledons can begin photosynthesis. Establishment and maintenance of isopogon and petrophile seedlings has consistently been reported as problematic by the study group, with slow growth, damping off issues and losses very common even when germination has been very successful. There are many losses when potting on emergent seedlings.

Small seeds in serotinous species like isopogons and petrophiles leads to small seedlings with small cotyledons. This leads to slower early growth; species with large seeds and large cotyledons have higher nutrient content and are associated with greater early growth reflecting more efficient transport of nutrients from cotyledons to seedling.

The slowest growth occurs in resprouter species which have a lignotuber as a response to fire. In general, resprouter seedlings tend to grow slower than reseeder seedlings. Shoot: root ratios of first-year seedlings and mature plants are higher for reseeders. Resprouters are developing a large, deeply penetrating root system and storing starch in root tissue whereas reseeders have a shallow, fibrous root system. The concentrations of essential nutrients in seedlings are reportedly no different between fire-response types.

Although species with smaller seeds are thought to be less drought hardy than those with larger seeds (more likely to survive the first summer drought), some isopogon and petrophile seedlings may be tougher than we might expect. Species with needle-like leaves minimise transpiration and tolerate dryness. Needle-like leaves are common in petrophiles, ranging from divided leaves (e.g. *P. seminuda* or *P. sessilis*), entire long leaves (e.g. *P. longifolia*), to entire short leaves (e.g. *P. ericifolia*). Another exception is *P. heterophylla*, where a trial found sowing in the middle of the dry season (January) resulted in higher establishment of seedlings compared with sowing at cooler times (April, end of dry season, or May, break of season).

The natural environment may provide a means to facilitate seedling fitness. In some plants, seeds contain fungi and bacteria which promotes seedling growth. This is common in agricultural crops and recent research in banksias has discovered a diverse range of fungal endophyte species, the fungi growing on the outside of cones, also growing inside the seed. The fungi were different in different species and were not present in every seed. It is not clear how microbes get into seeds and more research is needed.

Seeds are often released into a post-fire environment that contains active smoke chemicals and this may improve seedling fitness. The survival rates of post-fire seedlings growing in a bed of ash from a fire are generally higher although in isopogons and petrophiles this seems to vary by species. Myerscough et. al. (2001) report that an ash bed helped *P. pulchella* seedling establishment but not *I. anemonifolius*. Yearsley, Fowler and Ye (2018) found smokewater treatment of fruits enhanced seedling fitness in *P. filifolia* (greater shoot length) and *I. divergens* (greater root length). The benefit was confined to only two species of six tested (the others which showed no seedling benefits were *I. dubius*, *I.* sp (probably *I. spathulatus*), *P. anceps* and *P. drummondii*) indicating that any benefit is likely to be species-specific.

The role of water in promoting seedling fitness in not as clear as it is for germination. SG members report that too much moisture is a problem at this stage of development. This may be more so in species with needle-like leaves given the drought tolerance of such seedlings discussed above. The type of water could be relevant, with SG member Alex George reporting higher survival rates for seedlings watered with rainfall. Town water is likely to have a different chemical makeup; processed drinking water intentionally has minimal nitrates and can also be alkaline which makes nutrients less available to plants. Rain is rumoured to be a natural fertiliser, dissolving nitrogen from the air to deliver to plants, but only thunderstorms can form nitrates via electrical activity which combines atmospheric nitrogen and oxygen.

With such small seeds and seedlings in isopogons and petrophiles, adding nutrients may be particularly important for seedling fitness. It may be the nutrients in a bed of ash that promote growth in seedlings of P. pulchella as discussed above. At the WA Threatened Flora Seed Centre seedbank, gibberellic acid, a hormone, is used in the growing media of agar. In a 1997 study where he raised 810 seedlings of native plants, CSIRO Senior Research Chemist Kevin Handreck and volunteers sowed seeds in a potting mix which had ample supplies of all trace elements

to which he added nitrogen and potassium via a zero-phosphorus slow-release fertiliser. Proteaceae are notoriously susceptible to phosphorus and the general advice is to avoid phosphorous in fertilisers for these plants. Handreck subjected the trial plants to a range of phosphorous levels and compared the results to a zero-phosphorous control group, and included many Proteaceae species, including 15 species of isopogons and petrophiles. The trial showed that some Australian plant seedlings, isopogons and petrophiles included, benefit from the addition of phosphorous. Nine species showed greater growth with phosphorous with no ill effects (*I. ceratophyllus, I. dubius, P. canescens, P. carduacea, P. diversifolia, P. fastigiata, P. heterophylla, P. longifolia,* and *P. serruriae*). Seven species showed phosphorous toxicity of varying levels (including *I. alcicornis, I. axillaris, I. formosus, P. drummondii,* and *P. ericifolia*). At either end of the spectrum in this group were *Isopogon anethifolius* which showed slight toxicity symptoms at the highest phosphorous level but the largest healthy plants at the second-highest phosphorous level, and *Petrophile sessilis* which only thrived in zero-phosphorous conditions.

### Growing a new canopy seed bank

A new canopy seed store accumulates seed as a plant grows over time. A seedbank is vital to ensure the survival of the species population or the species itself in the event of plant or population death from fire or other cause. Some species have additional backup in the form of a lignotuber from which they can resprout, enabling adult plants to survive events which kill reseder species.

The first step is for newly established plants to grow into adult plants with mature seeds, to reach reproductive maturity. This is the earliest point at which fruit initiation is worthwhile and replacement would be possible. Up to this point a plant is in the juvenile period. During this time, a plant grows enough to be able to produce flowers which result in pollination and fruit set. Next, the seeds inside the new fruits in the cone need time to mature and become germinable. Compared to other serotinous eastern species, *P. pulchella* and *P. sessilis* have slow maturing cones (up to a year).

The information available on seedbank stages in isopogon and petrophiles species is patchy and comes from studies of the impact of different fire frequencies on serotinous species. The table below collates information from many different sources and is indicative only. Note that *P. pulchella* and *P. sessilis* are eastern reseder species. The only resprouting species explicitly included in this table is *I. anemonifolius*.

| Stage                       | Estimate  | Comments  |  |  |  |  |
|-----------------------------|---|---|--|--|--|--|
| JUVENILE PERIOD             |   |   |  |  |  |  |
| First flower production and | 2-3 years 2 years for WA northern sandplains species; 2 years for |   |  |  |  |  |
| fruit set                   |   | Darling Range jarrah forest species (90%), 100% within 3              |  |  |  |  |
|                             |   | years).   |  |  |  |  |
|                             | 4-5 years   | P. pulchella  |  |  |  |  |
| Seed development and        | 6-10 months   | Many species, particularly western                                    |  |  |  |  |
| maturation                  | 8-12 months   | P. sessilis and P. pulchella  |  |  |  |  |
| Reproductive maturity       | 3 + years   | WA species  |  |  |  |  |
|                             | 5-9 years   | P. pulchella  |  |  |  |  |
| CONE/SEED ACCUMULATION      |   |   |  |  |  |  |
| Sufficient to re-establish  | Over 6 years  | In reseeders in southwestern WA plant communities, 2-3                |  |  |  |  |
| population                  |   | times the time to flowering.  |  |  |  |  |
|                             | 8-10 years  | P. pulchella  |  |  |  |  |
|                             | Over 13   | <i>I. anemonifolius</i> has a smaller canopy store and its lignotuber |  |  |  |  |
|                             | years   | needs 13 years to develop.  |  |  |  |  |
| Maximum seed bank           | 18-20 years   | P. pulchella  |  |  |  |  |

Seedbank stages of newly established isopogon and petrophile plants: what we know so far

The duration of the juvenile period varies among serotinous species according to habitat, soils and regions. For example, juvenile periods are shorter in southwestern WA plant communities but longer in eastern cool regions like the Blue Mountains. After the juvenile period, cones really start to accumulate. As the volume of each plant's canopy increases, the number of cones increases exponentially each year in reseder species like *P. pulchella* and more

slowly in resprouter species. At some point, there are sufficient germinable seeds to re-establish the population to avoid possible decline should a fire or other destructive event occur. For resprouters, this is where a lignotuber really makes a difference to the chances of survival. If the plant is newly established, it is much more vulnerable to destructive events than reseeder species because its seedbank is smaller (due to investing resources in developing a lignotuber) but its lignotuber is not yet mature enough to be able to resprout. It takes much longer to grow a lignotuber than it does to grow a reseeder seedbank. However, if it is an adult resprouter (regardless of its current size) it will survive although it may not be able to recruit new juveniles due to its small seed bank.

Seedbanks keep growing in the absence of threats like fire, long after they reach sufficient numbers to re-establish. In reseeders like *P. pulchella*, the seedbank is estimated to reach a peak at 18-20 years. In western reseeder species, this period could be much shorter. Growth dynamics vary across species and habitats and regions. For example, *P. pulchella* is thought to have slower dynamics (slower growth, rates of maturation and longer juvenile periods) in the Blue Mountains than in coastal sites. Sydney species grown in South Africa have faster growth dynamics than near Sydney.

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## **Financial Report**

| Total 31/10/2022 | \$1,871.56               |  |  |
|------------------|--------------------------|--|--|
|                  | Bank balance \$1,768.12  |  |  |
|                  | Cash on hand \$103.44    |  |  |
| Donations/income | \$110.00                 |  |  |
|                  | Paddy Lightfoot \$100.00 |  |  |
|                  | Plant sales \$10.00      |  |  |
| Total 10/04/2023 | \$1,981.56               |  |  |
|                  | Bank balance \$1,878.12  |  |  |
|                  | Cash on hand \$103.44    |  |  |

Donations are welcome

ANPSA Isopogon & Petrophile Study Gr Bendigo Bank BSB 633-000 Acct 156858730