

# the Propagator



International Plant Propagators' Society <http://aus.ipps.org/>  
Australian Region - Newsletter Autumn 2024 - No: 78



## COUNTDOWN TO BALLINA

**"Whatever good things we build end up building us."**

**Jim Rohn**

As we officially roll into autumn, the countdown to this year's IPPS conference in Ballina is well and truly underway and the month of May will be here before any of us know it. So, if you haven't secured your spot, now is the time to do it.

It was with mixed feelings that I realised I will, unfortunately, miss this year's event. These conferences have always given me a sense of pride in IPPS and offer invaluable insights into propagation and our industry, so it will be disappointing to miss. However, the reason being that my wife and I have a new daughter due the week of the conference, is cause for celebration. I guess I can also take some solace from having had a chance to attend the mini-conference held to promote the society in South Australia about this time last year, where a great time was had by all, and participants were offered guest speakers, arboretum and nursery tours and a sausage sizzle to boot. It was no gala dinner, but everyone left with full stomachs and propagation front of mind.

It is with mixed feelings also, that I have decided to resign from the newsletter editor role and IPPS writer for Hort Journal Australia's contributions. I've enjoyed putting together 'the Propagator' each season and the feedback I have received is that it has been enjoyed by members too; so, I look forward to seeing what comes from a fresh pair of eyes and I am grateful for having had the opportunity.

The privilege of writing for Hort Journal Australia has now spanned close to ten years and has passed in the blink of an eye. While there have been times when the deadlines would sneak up on me and I'd have to squeeze writing into an already busy schedule, writing for the magazine

has undoubtedly made me a better propagator and lecturer; I think, collectively, the research involved and wordcount produced over the years could almost equate to a PhD in some shape or form and I have loved it and working with Karen Smith and Gabe Mustafa.

Now though, on the cusp of having two children under three, it's time for me to step back and allow a fresh pen to represent IPPS Australia. There are some exciting candidates among our members too, you just need to look back over the last few issues of 'the Propagator' where there have been some outstanding article contributions in recent releases.

Announcements aside, it is time to delve further into this issue of 'the Propagator' where, in the pages ahead, you'll hear from Bruce Higgs on his leadership movements; Tony VanderStaay reports on the international conference in South Africa and we delve into breaking seed dormancy, fungal associations of subterranean orchids, tissue culture and more.

Signing off on one last issue, Dan Austin – Editor.



*Edinburgh Parks Nursery staff Victoria Watkin, Alison Annells and Isabella Capurso at the SA mini-conference.*

*Image: Dan Austin*

### THIS ISSUE

- President's Report**
- Overcoming Native Seed Dormancy**
- International Director's Report**
- Fungi and Plants – Who's using Who?**
- Seeking New International Connections**

- A Message from the Proceedings' Editor**
- Tissue Culture Technology and Applications**
- Meet Nosipho Ndlovu**
- Propagator or Grower - News for You**
- Executive Officer's Report**

## President's Report

This is my final report to members in our newsletter as your regional President. I look back fondly on being encouraged to attend the 2001 Canberra conference and joining in 2002 in Melbourne, coinciding with MIFGS. I became newsletter editor at Mildura in 2005 and have attended conferences since joining.



## Overcoming Native Seed Dormancy

Dan Austin  
Lecturer/Author  
TAFESA

Australia is a unique country. Girt by sea as it were, we occupy a land that has had ample opportunity to diversify in species since forming portions of the mega continents of the past. This diversity has been driven by isolated plants and animals adapting to fill niche environments otherwise unoccupied and free of competition. As conditions changed and the continent matured, plants adapted and evolved to handle the varying conditions in all sorts of ways. In the tropics where conditions are moist, some plants have developed huge leaves that allow them to quickly disperse excess moisture and maximise photosynthesis under the thickly shaded canopy of the rainforest. While in areas of Australia that have become drier, some plants have evolved without leaves altogether to conserve valuable moisture in desert environments.



I've also had the privilege to be a part of the board since Brisbane in 2006. Through that time, I have seen the implementation of some tremendous initiatives such as our Six-pack, and shared with many people as often as I could. I intend to have another two years assisting the board as International Director though and assisting this great society of plants-people into the future. Conferences have provided a great opportunity to learn and take back ideas for our business as well as many holidays across the country and beyond.

So, reflecting on this time, I was saddened to be informed Dan Austin has found it necessary to stand down after this issue as our newsletter editor. He has been an encourager of many of our members over the years and has grown so much since being an early IPPS Southern African Exchange participant, and I have kept in touch over those years. Our Editorial Committee will be seeking expressions of interest in the near future for those who could assist in this role in the future. If you think that this is you please contact Pam Berryman or Clive Larkman.

I am looking forward to our Ballina conference in May. As I look back on the last few years and our 2022 strategic planning meeting which resulted in key points for future growth of our IPPS region (reinvigorated regional meetings and renewal of committee and structures), we have made some progress on these points and I hope we can continue these.

We now have an opportunity to renew our board with some vacancies at our AGM and we have a little time to get things into place for the next phase. Who could you recommend or approach to be part of this group? Your board is looking into a restructure of the board with the view of encouraging younger participation for future leaders. Nomination forms should be available soon from Pam Berryman.

Hoping we all have a great Autumn season.

Bruce Higgs

***Swainsona formosa* is an example of one of Australia's leguminous species that display seed dormancy.**  
**Image: Matt Coulter**

Along with these physical and visual adaptations, plants have evolved physiological processes that allow them to succeed in niche environments. One of the most common is observed at the very start of many plants' lives - at germination. It is rarely in a plant's best interest to have all its seed germinate at once. If the seedlings were to face any unfavourable environmental event, such as a drought or flood, an entire generation of offspring could be lost. So, instead, many plants have evolved so that their seeds achieve germinations staggered over a period of years.

This ability to remain inactive is known as seed dormancy and can allow seeds to remain viable for tens, hundreds and even thousands of years. Different plants have evolved diverse environmental triggers to activate dormant seed and induce germination. Some contain chemicals that must be leached from the seed through weathering and exposure to copious amounts of water, others must be exposed to periods of cold or hot temperature extremes. Some even need to pass through the digestive tracts of specific animals. However, in Australia there is one trigger plants have adapted to more than any other - fire.

As summer comes around in Australia, so do bushfires. They can be terrifying, sometimes catastrophic and we have all seen the devastation they can cause, but fires have played a part in Australia's landscape for as long as time has allowed. For many plants, there is no better time to germinate than after a fire. Ash has deposited a short supply of additional nutrients to the soil and competition for light and water has been reduced or eliminated. So, it is no wonder the seeds of many Australian species are reactive to the smoke and heat of bushfires.

Seed dormancy is a fantastic way to ensure plants do not put all their eggs in one seasonal basket and in nature, is an evolutionary adaptation that has allowed many species to thrive. However, for plant propagators, seed inactivity can be problematic, and seeds must be manipulated in some way to achieve germination and bypass any dormancy mechanisms. To do this, propagators first need to understand how it is, that each species' seed achieves dormancy.

Many cool climate and alpine species require a chilling period to simulate icy winters, as germinating before winter would lead to certain death for any young seedlings. Propagators achieve this chilling period by placing seed or other propagation material into refrigeration before sowing in processes known as stratification and vernalisation. Other plants including many of Australia's leguminous species have hard seed coats which require mechanical weathering or the heat of fire to weaken them enough for water to be able to penetrate to the waiting embryo inside.

There are various techniques propagators use to get past this mechanism of dormancy. Some species respond to hot water treatments where seed is added to water one minute after boiling and allowed to soak (for several hours in many cases). Other species require mechanical manipulation of the seed coat. In a process known as scarification, hard seed coats can be physically weakened to allow for water penetration. This can be a simple process like rubbing seeds between two sheets of abrasive sandpaper, or for more difficult species, can be quite challenging and require the use of a scalpel and microscope.



***Swainsona greyana* is best propagated by utilising seed scarification with a scalpel and microscope.**

**Image:** Matt Coulter

The dormancy of other seeds can be overcome through chemical interactions. Plant hormones in the gibberellin group act as chemical messengers within seeds and are thought to induce the metabolism of starches, signalling germination in many species. Gibberellic acid treatment of seed has long been part of the propagator's bag of tricks for difficult-to-germinate species.

For some plants it is not the heat of a bushfire that activates dormant seed, but the chemical compounds found in the subsequent smoke and ash. Karrikins are a group of compounds produced when heating and burning carbohydrates such as cellulose and have been found to act as effective plant growth regulators. Karrikins produced by bushfires are washed into soil by rains following fires where dormant seeds reside, sending the seeds a chemical 'wake up call'. For propagators, this knowledge has been invaluable in the propagation of many difficult-to-germinate Australian species. Exposing seed to karrikins has been achieved in various ways from placing seed into smoking tents, to condensing smoke into a liquid or 'smoke water' in which seed can be soaked. More recently, however, karrikins have been synthesised, allowing for the development of all sorts of products from smoke water tablets to smoke-infused vermiculite.

Plant species without seed dormancy mechanisms only require water, oxygen, the correct temperature, and a favourable location to achieve germination. While in other



species, germination is determined by contributing factors of moisture, temperature, light, chemicals and physical action. To make things more complicated many plants require a mixture of conditions involving multiple treatments to initiate germination.

Ours is a challenging and rewarding field, so if you have had trouble with propagating native seed or plant material in the past, make sure you identify the species and research any pre-treatments required or better yet pick the brains of an experienced IPPS propagator at this year's conference in Ballina.



**From top to bottom: Seed soaking in synthetic smoke water, regrowth of understory after karrikins produced by a bushfire activate the seedbank in soil in Tasmania's Central Highlands.**

**Image:** Dan Austin



## 52nd IPPS Conference 2024



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more information***

## International Director's Report

Hello fellow members,

I was privileged to attend the international South African tour last month, where there were 39 starters on the tour, with 5 from our region, 7 from New Zealand, 3 from Europe, 16 from the Americas, and the rest from South Africa. The food and wine were good, the attractions and nurseries we visited were great and the company we experienced was fantastic.



for at least three years. The new international president is Grant Hayman from New Zealand region.

During this tour, the international board met on two occasions with a small third meeting on Day 5 of the tour. On this occasion, we met for over 10 hours of face-to-face discussion. The most controversial item on the agenda was the proposed name change, and after quite a long discussion it was agreed that the arguments for and against would be circulated to all regions. The biggest item in the agenda was a review of the operating manual of the society from an international point of view, some of the policies dated back to 1998, and have never been updated, fortunately, Tom and Donna Fare (treasurer) had the foresight to pre-empt the situation and had done a lot of the necessary updating of the policies, even so, it took the best part of 6 hours to go through the policies and update them.

The board elected a new international chairperson, Tom Saunders, from the southern region, and he will be there

Grant outlined the next international tour in New Zealand set for April/May 2025, concentrating on the north island only with limited bus travel, starting in Auckland for the first 2 days, visiting nurseries around the greater Auckland area then moving east and south ending up in New Plymouth, and as with all tours there will be plenty of surprises.

Meeting fellow members from different regions gives you insights into issues that don't generally worry us, the average basic wage in South Africa was 27 rand per day (\$3.00 Aussie), yet prices in the supermarkets and fuel were very compatible with our prices, so yes, they have very cheap labour but also huge issues in getting people to work consistently. how people survive is amazing and there seems to be a fairly positive horticultural industry happening there. See you in Ballina. Cheers.

Tony VanderStaay



**From top to bottom: Joshua Taylor joined the group representing the IPPS Australian Region as part of his South African Exchange Programme, members on the international tour.**

**Images: Brienne Gluvna Arthur**

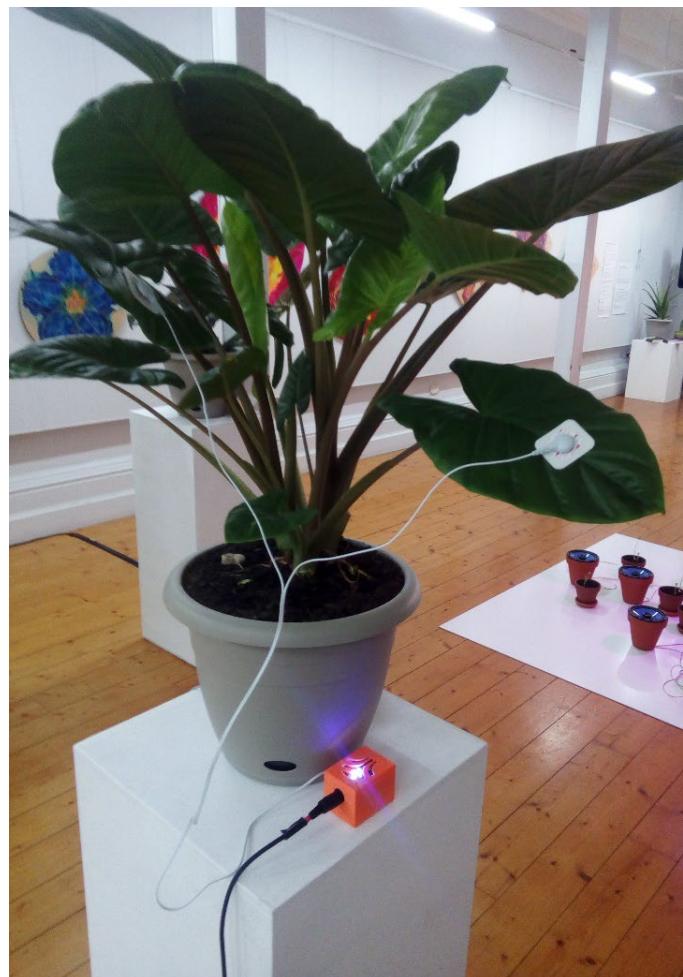
## Fungi and Plants – Who's using Who?

Dan Austin  
Lecturer/Author  
TAFESA

When it comes to studying the natural world, you quickly learn that the more you know, the more you know you don't know, and plants are no exception. For every plant species or process known to science, we know a myriad are not, and even for those deemed to be understood, countless questions still remain.

Many such questions arise when looking at how plants interact and even communicate with the world around them. Though often difficult to observe in our timescale, plant responses to the environment and relationships with the animal kingdom have been extensively researched and documented but there is one field we are often quite literally 'in the dark' about.

Whether parasitic, symbiotic or coincidentally beneficial, the relationships between plants and fungi have existed for millennia but by nature, their interactions have largely gone unseen. Comparable to an illicit relationship kept hidden behind closed doors and only coming to light



when a baby is on the way, often it is only when seeing a fungal fruiting body, we become aware of its existence. So, it is no wonder research in the area has been relatively slow.

Many parasitic fungi are well researched though, through necessity, as they have posed a threat to our food security or commerce in the ornamental industry. Living on the surface of (ectoparasitic) or within plant tissues (endoparasitic), parasitic fungi exist at the expense of the host plant. They draw nutrition and water from the plant, reducing its vigour, yields and in the worst case, leading to the death of the host.

However, only just coming to light is the knowledge that a handful of plants can turn the tables and parasitise fungi!

*Rhizanthella gardneri* is an amazing plant for a multitude of reasons - when being a subterranean orchid that flowers underground and is pollinated by termites, is not the most interesting thing about a plant, amazing is a pretty appropriate term. This Western Australian native is what is known as a myco-heterotroph, wholly parasitic and unable to photosynthesise, it extracts sugars from both



From top to bottom: Experiments translating plant electronic signals into audible sound, *Rhizanthella gardneri* a subterranean orchid native to Western Australia..

Image 1: Dan Austin, Image 2: <https://www.flickr.com/people/63479603@N00>

broombush (*Melaleuca uncinata*) and mycorrhizal fungi present in the soil including *Thanatephorus gardneri* and *Ceratobasidium* spp.

Mycorrhizal fungi are another group of fungi highly involved with plants but rather than parasitising their hosts, mycorrhizae form a mutually beneficial 'give and take' relationship with plants. Once connected to the host's roots, the microscopic strands (hyphae) forming the body of mycorrhizal fungi, massively increase the surface area available for plants to extract nutrients and water from the soil. The plant, in return, can photosynthesise and produce the valuable sugars and energy needed for both its own growth and its associated mycorrhizae.

Some mycorrhizal relationships are highly exclusive to the plant and fungi species involved, as with *Melaleuca uncinata*. Propagators working with rare and difficult to grow plant species will sometimes collect soil at the site they collect their seed to inoculate their growing media back

at the nurseries. At times propagators have even needed to extract mycorrhizae from plant tissues *in situ* to grow highly dependant plants. Other plants readily form cosmopolitan relationships between a range of species of fungi and vice versa. This has allowed for the commercialisation of mycorrhizal inoculants for plant and soil health which are available as tablets or powders containing multiple species of indiscriminate fungi including species of *Glomis*, *Pisolithus*, *Scleroderma* and *Trichoderma*.

The importance of mycorrhizae in the plant kingdom cannot be overstated and without them, many plants would not even germinate. Several terrestrial orchids produce seed that is so minuscule, it is virtually incomplete and lacks the stored energy required for germination. It is not until mycorrhizae colonise these seeds and provide valuable sugars that germination can even take place!

From the smallest seed to the largest forest, mycorrhizae are present.



***Beauveria bassiana* fungus erupting from the joints of a parasitised beetle.**

Image: Sourced by Dan Austin with permission

It is in these larger masses that the true extent of the hidden secrets of plant and fungi relationships are really coming to light. With the development of technology that has allowed scientists to radiolabel carbon and track its movement in organisms, an amazing relationship has been revealed. In healthy forests, plants are completely interconnected by mycorrhizal networks and through these networks, actually share resources between plants and even between differing plant species! Plants communicate using mycorrhizae and it makes sense. How else is a young sapling growing in the heavy shade of a forest canopy going to make it without sunlight, if not from a little resource sharing from the elders?

Those of you who were present at last year's conference would also have learned through Elliot Akintola's Rod Tallis Award presentation, that plants also have the ability to 'call out' to mycorrhizal fungi to assist with phosphorus uptake, when they sense levels in soil are low through a process using volatile organic compound signalling – amazing!

The idea of plant communication is not new and 'The Secret Life of Plants' by Peter Tompkins and Christopher Bird explored the idea back in the seventies and as a curio, it has become a best seller since. It's been so popular, in fact, it has been the catalyst for much research into plant

electrical signals and their potential as a form of communication and has even inspired software that translates these signals into audible sound. Perhaps not useful as a translation in a conventional sense but interesting nonetheless.

Plants benefit from fungi incidentally as well. Saprophytic fungi may not have an active relationship with living plants but they break down dead organic matter which can then be taken up again by plants as a valuable food source. Other fungi including *Beauveria bassiana*, *Cordyceps* spp. and *Metarhizium* spp. are entomopathogenic and parasitic on various insects. Some of these have even been commercialised as biological insecticides for grasshoppers and locusts in particular.

Think of the active role that the organisms we consider inanimate are playing day to day and you get a whole new appreciation of the humble mushroom. It is easy to see we have barely scratched the surface of understanding the natural world.

We may never solve all the questions of life on Earth but hopefully in the years ahead we will at least solve a few more about life in the rhizosphere.

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## Seeking New International Connections

David Hancock and Dermot Molloy

IPPS Board and long-time members

Active Board members Dermot Molloy and David Hancock took a stopover in Mauritius on the way to the recent international meet in South Africa. With hormones and smoked product giveaways in hand, they held meetings with management and growing staff at Mauritius Wildlife Fund and Endemika nursery.

The MWF discussed their plans for an additional nursery to support the planned restoration of a disturbed area adjacent to the Mondrian Conservation Reserve. Mondrian is a 5-hectare area on the crown of the Vacoas Ridge overlooking the Magenta Valley. The reserve represents a valuable remnant and functional forest ecosystem and a haven for threatened plants and animals.

The Endemika nursery is located near Grand Baie in the north of the island country and provides a wide range of



local and introduced species. The Endemika connections have done some fine work in restoring mangrove wetlands. The propagation facilities are very basic and the lack of industry expertise on Mauritius was evident.



**From top to bottom: David Hancock offerin advice, Dermot Molloy with propagators from Mauritius Wildlife.**  
**Images: Dermot Molloy, David Hancock**



Dermot & David offered advice that was eagerly accepted and it was agreed that follow-up assistance would be provided to assist their growing success.

Two other conservation areas were intended for visitation, but the arrival of a cyclone prevented these visits until another time. In summary, some sound contacts were established and membership of IPPS can be expected likely through the South Africa division.

### A Message from the Proceedings' Editor

The philosophy of the International Plant Propagators' Society is to "seek and share". The major way information is shared with the membership is through the publication of manuscripts associated with oral and poster presentations at the annual meetings for each IPPS region. We have a history of publishing proceedings papers dating back to 1951. All papers published since that date are now available to members electronically on the IPPS website. Thank you for adding to this legacy of production and propagation information by submitting a written version of your presentation.

Manuscripts will be accepted any time before the annual conference. Please submit by e-mail to the Editor Dr Ranjith Pathirana ( [Ranjith.Pathirana@Adelaide.edu.au](mailto:Ranjith.Pathirana@Adelaide.edu.au) ) as a Word document. The manuscript is required within two weeks, following the annual conference (10th June 2024). You can submit the electronic version of the manuscript during the annual conference. There are no page limits for your manuscript, but papers are generally 3-8 printed pages long. Including color images in the manuscript is encouraged and links to posted videos can also be included. Manuscripts generally follow one of the following formats with major headings:

Research paper format should follow a standard Title, Authors, Keywords, Summary (150-250 words), Introduction, Materials and Methods, Results and

Discussion, Conclusion, Acknowledgements (if any) and Literature Cited.

Production paper format can be more flexible. The suggested headings would be Title, Authors, Keywords, Summary (150–250words), Introduction, Production/Propagation Methods, Key Innovations (or Standard Practices), Acknowledgements (if any) and Literature Cited when appropriate. Review papers should have a Title, Authors, Keywords, Summary, Introduction, and subheadings for key points in the review followed by Literature Cited.

Please ensure that all tables, diagrams, and images are clearly labelled and referred to in the manuscript. Do not include any copyrighted content unless you have prior approval. Please sign and return the copyright document along with the manuscript. Links to sample manuscripts are available at [IPPS.org](http://IPPS.org) or I can share with you.

Keep in mind these points when preparing your paper:

- ❖ The published paper does not have to be identical to your talk. The published form should be representative of your presentation, may contain greater detail, and be more structured.
- ❖ A text-based file is needed for publication, not the PowerPoint.
- ❖ Please indicate the word processing program used during preparation as this is critical information if problems arise in opening the file.
- ❖ Figures constructed in Excel must be created only with black lines. Colors other than black often do not print well when converted to black and white. Please do not use colored backgrounds such as green, blue, red, etc.
- ❖ Images for publication should be at least 300 DPI and are encouraged.

Thank you for being an important part of our annual conference program. Please contact me if you have any questions and I look forward to seeing you at Ballina!

Regards,

**Dr Ranjith Pathirana (Editor, IPPS Australia)**  
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***Mauritius growers working with limited resources.***

**Image: David Hancock**

## Tissue Culture Technology and Applications

Plant tissue culture (in vitro culture) constitutes a critical component in plant biotechnology and refers to the culture of plant cells, tissues or organs on a nutrient medium under aseptic (sterile) conditions. During the growth in vitro, we control temperature, humidity, light intensity, light cycle and manipulate media components. The cultures are either maintained in highly controlled growth chambers or culture rooms in liquid or solid media. Although widely used application is micropropagation, there are many other applications such as virus eradication from infected clones, crop improvement, conservation and producing biopharmaceuticals, food ingredients, cosmetics, flavours, dietary supplements, fragrances, and bio-stimulants. In this series of articles, I will discuss different applications of plant tissue culture, and this part is devoted to micropropagation – propagation of clonal plants through tissue culture. I will use examples from my own work as far as possible, so I don't infringe upon copyright of others, and because I am more familiar with the response of plants I have worked with.

In tissue culture we grow plant cells or tissues in artificial culture media, whose proportion and concentration of elements vary depending on the objective and plant species. We supply major and minor elements required for growth and development in the form of inorganic salts, for example, magnesium sulphate to supply magnesium and sulphur. There are many different media available commercially for different species and we need to test different media for less studied species.

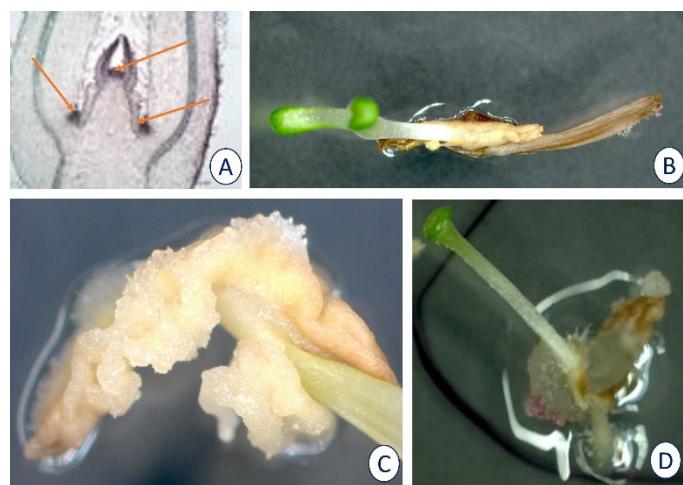
Plants or cells growing in artificial media achieve none or very little photosynthesis. Even if leaves are present, their stomata are always open and non-functional. So, we need to supply carbon through the media as the plants in culture can't use carbon dioxide from the atmosphere of the culture vessel to fix carbon. The main carbon source is sucrose, but often we use other sugars such as maltose, fructose, glucose etc.

Plant growth regulators are an important component in culture media, and we can drive the growth and development to our requirements by changing the proportions of auxins, cytokinins, abscisic acid, gibberellic acid etc. Additionally, we have to give support to plants by making media solid using gelling agents such as agar or gellan gum, unless we use liquid cultures.

## WHY ARE PLANT CELLS AND TISSUES AMENABLE TO TISSUE CULTURE?

The plants grow and develop throughout their life and for this they have dividing meristematic cells that we can always use to initiate cultures (Fig. 1A). In addition to the meristematic tissue in apices and axillary buds, vascular cambium (a layer of cells between primary xylem and phloem) and cork cambium (a major portion of the bark of woody plants) also have meristematic tissue. Dividing meristematic cells are easier to manipulate than differentiated cells. However, unlike most of animal cells, plant cells have totipotency and pluripotency. Totipotency is the ability of any living plant cell to revert to a meristematic (dividing) state, given the right environmental cues and then produce a complete plant. Pluripotency is the ability to regenerate an organ or tissue from a living cell. Thus, a differentiated plant cell can return to a meristematic state and then differentiate to produce tissues and organs that have specialised cells. This latter process is called de-differentiation.

For an example, we can get an anther to produce a haploid plant (plant with a single set of chromosomes) directly using low auxin and high cytokinin in media (Fig. 1B) and this is an example of direct regeneration. When we produce plants from cells through an intervening callus phase by manipulating plant growth regulators (Fig. 1C and D), the process is called indirect organogenesis. This is generally a two-step process. For clonal propagation direct organogenesis is preferred because when going through a callus phase, there are chances of mutation induction.



**Figure 1.** As plants continue to grow during their life, meristematic tissue (red arrows) is present for easy manipulation (A – meristems of shoot tip and axillary nodes in *Hibiscus rosa-sinensis*), Direct regeneration of a haploid plant from an anther of *Gentiana triflora* (B), indirect organogenesis (D) of the same species through an intervening callus phase (C). (From Pathirana et al. 2011. Plant Cell Reports, 30, 1055–1065).

The ratio of auxins to cytokinins invariably determines structural organisation in vitro, a concept published as early as 1957 (Skoog and Miller, 1957). Since then, we have learnt much more about organogenesis in plants. The differentiation of cells into organs or undifferentiated callus is guided by this ratio as well as concentrations and types of growth regulators used in media. This is illustrated in Fig. 2.



**Figure 2. Determination of structural organisation of plants in vitro as affected by ratio of auxin/cytokinin**

### CLONAL PROPAGATION THROUGH TISSUE CULTURE

Clonal propagation in tissue culture, technically called micropropagation, is the most widely used and widely known application among the many in vitro technologies. According to [P&S Intelligence](#) (2024) the global micropropagation market stood at US\$ 1.28 billion in 2022 and is expected to grow to US\$ 2.1 billion by 2030. Due to the growing interest of the young population in indoor plants. The largest driver of this growth will be the increased demand for orchids and other indoor plants. Additionally, the wide use of ornamental plants in the decoration of commercial facilities such as hotels, airports, restaurants and office spaces adds to this demand.

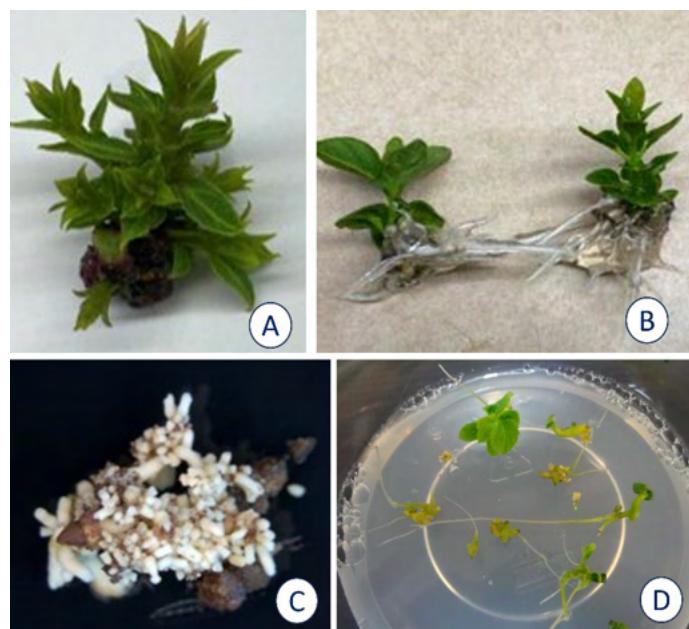
The largest micropropagation facilities can be found in China, India, Thailand and South American countries where labor is relatively inexpensive as this is a labor-intensive process and automation is difficult and costly. Micropropagation is also used in large-scale propagation of banana, pineapples, potato, *Pinus radiata* (and many forest species) and many floricultural and horticultural crops, as well as for medicinal plants including medicinal cannabis (with high tetrahydrocannabinol content, which is the psychoactive chemical), with many countries legalising its medicinal use.

The growth of the micropropagation industry is boosted by the demand exceeding the current production capacity. It is estimated that the global demand for healthy, clean and uniform planting material is about 16 trillion equating to US\$ 4 trillion, whereas only 1.5 - 2 billion plants are produced through micropropagation (van

Horen, 2019). Undoubtedly micropropagation is the leading technology that can deliver quality plants throughout the year. Despite tissue culture production facilities being located mainly in developing countries, major companies have installed acclimation facilities in more developed areas for tissue-cultured plants they buy, thus reducing costs.

Clonal multiplication in tissue culture is achieved through different pathways, and the main approaches are direct organogenesis as demonstrated in our recent experiments with *Corymbia* spp. (Fig. 3 A and B), indirect organogenesis via a callus phase (Fig. 1 C and D) or through somatic embryogenesis (Fig. 3 C and D). Direct organogenesis involves the use of plant growth regulators, particularly cytokinins, to induce the growth of existing shoot primordia in the shoot apex and axillary buds, resulting in multiple shoots that can be separated and further multiplied. For indirect organogenesis, we first produce a callus tissue with undifferentiated cells from which large numbers of plantlets can be generated.

Somatic embryogenesis involves producing bipolar structures resembling zygotic embryos from non-reproductive, somatic tissue such as leaves (Fig. 3 C), petioles, cotyledons or even roots, without a vascular connection with original tissue. These then go through a maturation process and can be germinated, usually by increasing the concentration of auxins in growth media or in hormone-free media (Fig. 3 B, 3 D).



**Figure 3. *Corymbia* micropropagation through direct organogenesis (A) Multiple shoot formation from a shoot tip, (B) rooting of separated microshoots. Production of somatic embryos of kiwifruit (C) and their germination (D) (From Pathirana et al. 2016. Acta Hortic. 1127, 217-222)**

Somatic embryos differ from sexually produced zygotic embryos by the absence of a seed coat, although they have the embryonic axis with radicle and plumule including cotyledons. The somatic embryos, unlike zygotic embryos, do not have tolerance to desiccation. Therefore, they cannot be handled like dry seeds. Zygotic seeds of many species can be dried to 6-8 % moisture content for storage and used for planting, retaining their viability at that low moisture content. However, somatic embryos cannot be dried. Studying the process of acquisition of desiccation tolerance in fertilized ovules of alfalfa (*Medicago sativa*), scientists at Guelph University in Canada managed to mimic this process in somatic embryos. Adding abscisic acid in correct concentration to media at the cotyledonary stage of embryo development was crucial (Senaratna, 1989). Thus, the possibility of imparting desiccation tolerance to somatic embryos exists, but the challenge is synchronizing the process of embryo development (to apply abscisic acid at cotyledonary stage) in tissue culture systems. Therefore, most of the somatic embryo-based systems of micropropagation rely on using alginate beads to encapsulate the embryos for short-term storage or directly germinating the embryos to produce plants.

Synchronized production of somatic embryos in suspension cultures, maturing in on solid media followed by drying and encapsulating in an inert substance such as clay with incorporation of more nutrients has the potential to revolutionize the clonal propagation industry.

The other popular method used in micropropagation is microtuber production in the case of tuber crops like potato, ulluco, yams etc. In major production areas of potato, microtubers are the preferred option for 'seed potato' production. Microtubers can be produced from single nodal cuttings in media with high sucrose (6 – 9 %) supplemented with cytokinins, mainly kinetin. The cultures are maintained in the dark for tuber production. Potato and ulluco (*Ullucus tuberoses* - a South American tuber crop) microtuber production in my work in New Zealand is presented in Figures 4 and 5, respectively. In the potato, we wanted to understand if the microtubers can be used to screen potato germplasm for cold-induced sweetening (CIS), a problem encountered in processing potato (Pathirana et al., 2008).

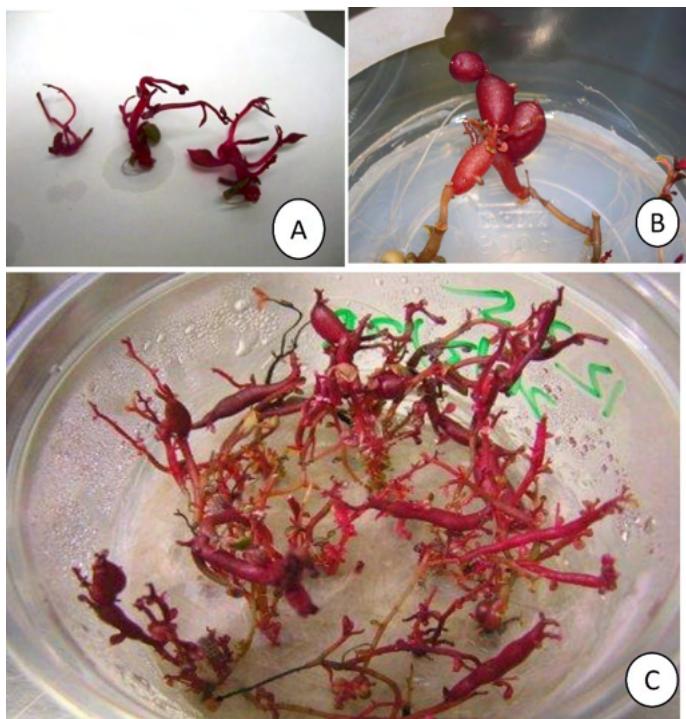
A rise in hexose sugar levels during cold storage of potato tubers results in a brown, bitter tasting and unmarketable product. This is caused by invertase enzyme activity in cold storage and this activity and hexose sugar production is different in different potato cultivars. A comparison of field-grown potatoes and microtubers showed that there is a good correlation in CIS and hence microtubers are a good model for selecting potato genotypes for processing. In ulluco, our objective was to produce diverse genotypes to adapt this new crop to New Zealand

conditions through mutagenesis (Pathirana et al., 2011). In vitro mutagenesis is an efficient way to produce large mutant populations and the ability of ulluco to produce microtubers is an advantage for the easy transfer to field conditions for screening the mutant populations. Our work resulted in mutants with traits such as early maturity, altered tuber morphology and colour, less geosmin (a component in ulluco tubers that imparts an 'earthy' taste) and altered leaf colour.



**Figure 4. Stages of microtuber production in potato. (A)** Tissue cultured plantlets in culture media for microtuber production, **(B)** Microtubers produced within 3-5 months, **(C)** Morphological diversity of microtubers from different cultivars - they resemble their counterpart field-grown tubers not only in colour and shape, but also in cold-induced sweetening (From Pathirana et al. 2008. Post Harvest Biol Technol, 49; 180-184)

Growing of mother plants in a disease- and stress-free environment is important to initiate cultures for all the methods. Optimally they should be sourced from a greenhouse. For this reason, we graft *Corymbia* hybrids of street appeal and use actively growing shoots in the greenhouse as explants (parts used in culture). One might ask why we don't propagate these selected hybrids by grafting. This is because of compatibility issues and the grafted plants not surviving long due to breaking at graft union (Pers Comm. Dr. Kate Delaporte, Curator, Waite Arboretum). Therefore, tissue culture is indispensable in cases like these.



**Figure 5. Production of microtubers of ulluco (*Ullucus tuberosus*) for a mutation breeding program. (A) Microshoots in culture media with high sucrose and cytokinin for microtuber production, (B and C) Microtubers produced within three months.**

The stages of micropagation through direct organogenesis include the initiation of cultures on proliferation media, multiplication (Fig. 3 A), rooting of microshoots (Fig. 3 B), exflasking and acclimation. For micropagation through direct organogenesis, only those parts of a plant that have meristematic tissue with shoot initials are used and cultured in media supplemented with a higher proportion of cytokinins than auxins (Fig. 2). This proportion is reversed for rooting of microshoots, and this aspect was recently discussed in a new breakthrough for cherimoya in this newsletter (Josekutty, 2023), where two auxins instead of one were used for rooting. Sometimes the cytokinin used influences subsequent rooting. Recently we have shown that the use of *meta*-Topolin (*mT* - a natural cytokinin first found in poplar – topola is the Polish word for poplar) in place of benzylamino purine (BAP) or zeatin helps rooting of a ‘difficult-to-root’ red kiwifruit (Saeiahagh et al., 2019). Lower hyperhydricity (a physiological disorder often found in tissue cultures) and low residual effect of *mT* are some reasons for this success.

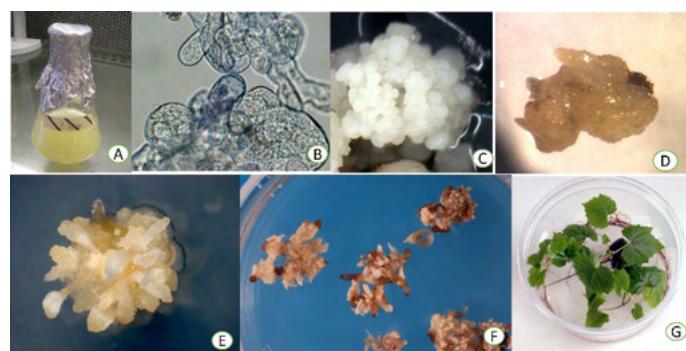
In general, rooting of many woody species is challenging compared to annuals and herbaceous species. To enhance rooting, supplementation of media with activated carbon, pulse treatment of microshoots with a high concentration of auxins (1-50 g/L; 2 – 30 min), incubation for several days in the dark, reduced mineral and sucrose content in media, as well as photoautotrophic

micropagation systems (systems mimicking natural growth conditions with higher light intensities, no sucrose supplementation but often using liquid cultures), have been used, and for each species, the methods need to be optimised. Some species, like blueberry, can be rooted in the greenhouse, without a rooting phase in tissue culture.

After the rooting phase, the plants are taken out from agar media, washed and planted in specialised potting mixes. These potting mixes are often autoclaved to reduce the infection of tender and vulnerable plantlets in the early stages of acclimation. The greenhouse acclimation of rooted microshoots is a challenge and was discussed in detail in a recent article in this newsletter and the reader is referred to it for details (Wightwick, 2023).

Micropagation through somatic embryogenesis has the potential for scaling up and automation of production of clonal plants. It is amenable to suspension cultures as shown in our work with kiwifruit (Pathirana et al. 2016) and grapevines (Pathirana and Carimi, 2023). If the process can be well tested and established, mass production for the nursery industry is a possibility as in the case of *Pinus radiata*, with scientists introducing machine learning algorithms to predict and select somatic embryos with high rates of germination success (Scion 2024).

Our recent attempt for scaling up grapevine somatic embryo production (in this case for in vitro mutation breeding) is given in Figure 6 showing different stages and steps, starting with the establishment of cell cultures with proembryogenic masses. The proembryos then go through globular, heart, torpedo and cotyledonary stages in dicots. Abscisic acid is generally used for maturation, and they can be germinated in solid media (Fig. 6).



**Figure 6. Somatic embryo production from suspension culture in grapevine. (A) suspension culture established from embryogenic calli of floral tissue, (B) Proembryogenic cell clusters, (C-E) different stages of somatic embryo development on solid media, (F) mature somatic embryos on germination media and (F) plants regenerated from somatic embryos. (From Pathirana and Carimi, 2023)**

Optimum conditions for germination and conversion of somatic embryos can vary widely depending on the plant species and embryo development. Culture media, sucrose concentration, light and temperature conditions, as well as hormone types and concentrations, need to be optimised for each species. Another advantage of somatic embryos is that they are easy to cryopreserve for long-term storage, a huge advantage for in vitro breeding and conservation as we have shown for kiwifruit (Pathirana et al 2016) and for an endangered grapevine species (Carimi et al 2016).

## FUTURE PERSPECTIVES

Reducing labour cost and increasing throughput in micro-propagation systems is the main challenge, as we can now micropropagate almost any plant species. This will significantly reduce the cost of plant material produced. For this, robotic systems that can be programmed to perform tasks such as subculturing and harvesting plantlets, automated liquid handling systems for accurately dispensing of culture media can be developed. Specialised culture vessels equipped with sensors and actuators can automate different stages of tissue culture, such as controlling temperature, humidity, gaseous content within the vessel and light conditions. Such vessels can also allow the monitoring and management of cultures remotely. To ensure quality control and traceability, barcoding and tracking systems can be incorporated easily in large-scale operations.

Image analysis and machine vision systems can be used to automate and streamline the monitoring of growth, development, and the health of plant material and to reject weak or underdeveloped plants and somatic embryos. These systems can be incorporated with feedback loops to optimise the process. High throughput screening platforms to test media formulations and environmental conditions for research into new species would help reduce cost of incorporating new genotypes, species in micro-propagation production lines. Suspension cultures and bioreactors can be improved to automate production systems and handle large quantities of plant material under controlled environments.

To the other extreme, increasing small-scale operations in rural areas with government subsidies can help meet local demand and fill the gaps in supply, particularly in niche markets for endangered or rare species and unique cultivars. Local governments can assist in organising training programs and providing educational tools, particularly those local councils already managing nurseries of their own.

In conclusion, tissue culture has immense potential to further increase mass clonal production in horticulture,

floriculture and forestry industries including industrial and medicinal crop species, as well as for conservation efforts. This author is happy to seek and share knowledge with industry partners in this area.

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## Meet Nosipho Ndlovu



My name is Nosipho Ndlovu, a passionate and aspiring horticulturist with a deep love for plants and the natural world. Growing up in a small rural town in KwaZulu Natal, South Africa, surrounded by lush gardens and farms, I developed an early fascination with plants and their growth cycles. My childhood was mostly spent exploring the outdoors and planting with my grandfather in his garden, which instilled a profound respect for the environment and gave me a desire to pursue a career in horticulture. My academic career began shortly after I matriculated in 2015 and went on to Durban University of Technology to study Horticultural Science.

I am in possession of a National Diploma and an Advanced Diploma in Horticulture from the Durban University of Technology. My research was on strategies towards a micropropagation protocol for the threatened *Gymnosporia woodii*. I was employed as a horticulture intern at SANBI Kirstenbosch National Botanical Gardens for 2 years, my duties included the redevelopment of garden sections with emphasis on hard and soft landscaping elements, conservation and propagation protocols for threatened plant species, maintenance plan updates, inventories, seed collection trips with the MSB populating popular articles and interpretation of garden beds for better visitor experience.

After completing my internship, I was offered a scholarship by SANBI which allowed me to pursue a Postgraduate Diploma in Conservation Horticulture at the Cape Peninsula University of Technology which I completed in 2023 with a research project focused on the effects of cutting technique and auxins on the rooting of *Diosma Haelkraalensis*, an endangered species of the fynbos biome. I am currently a registered MSc candidate in Conservation science.

I have a deep determination of exploring my interests in Horticulture which include learning about the different

plant propagation techniques, conservation and germination protocols, tissue culture, restoration as well as exploring various botanic gardens and institutions in my field. My ultimate goal as an aspiring horticulturist is to contribute to the advancement of sustainable Horticulture, promote biodiversity conservation, and empower communities to embrace the beauty and benefits of plants. With my passion, knowledge, and dedication, I hope to one day create a greener and more vibrant world for future generations to enjoy.

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## Executive Officer's Report

### IPPS Office

I have still been kept busy with 'day-to-day' running of the IPPS Office as well as following up on membership subscription renewals.



### Membership Subscription Renewal 2024

#### Membership Renewals for 2024 are now OVERDUE.

The Australian region is required to report to the International Board the number of membership subscriptions for 2024 this month.

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### Contact Detail Changes

To ensure Office records are kept 'up to date' I would appreciate it if Members could please notify me of any changed contact details. In particular, if you have changed telephone provider recently, please advise me of your new email address at [pam@ipps.org.au](mailto:pam@ipps.org.au). It is important that the Australian & International database records are kept 'up to date', otherwise you could be missing out on receiving information.

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