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NZ Plant Producers

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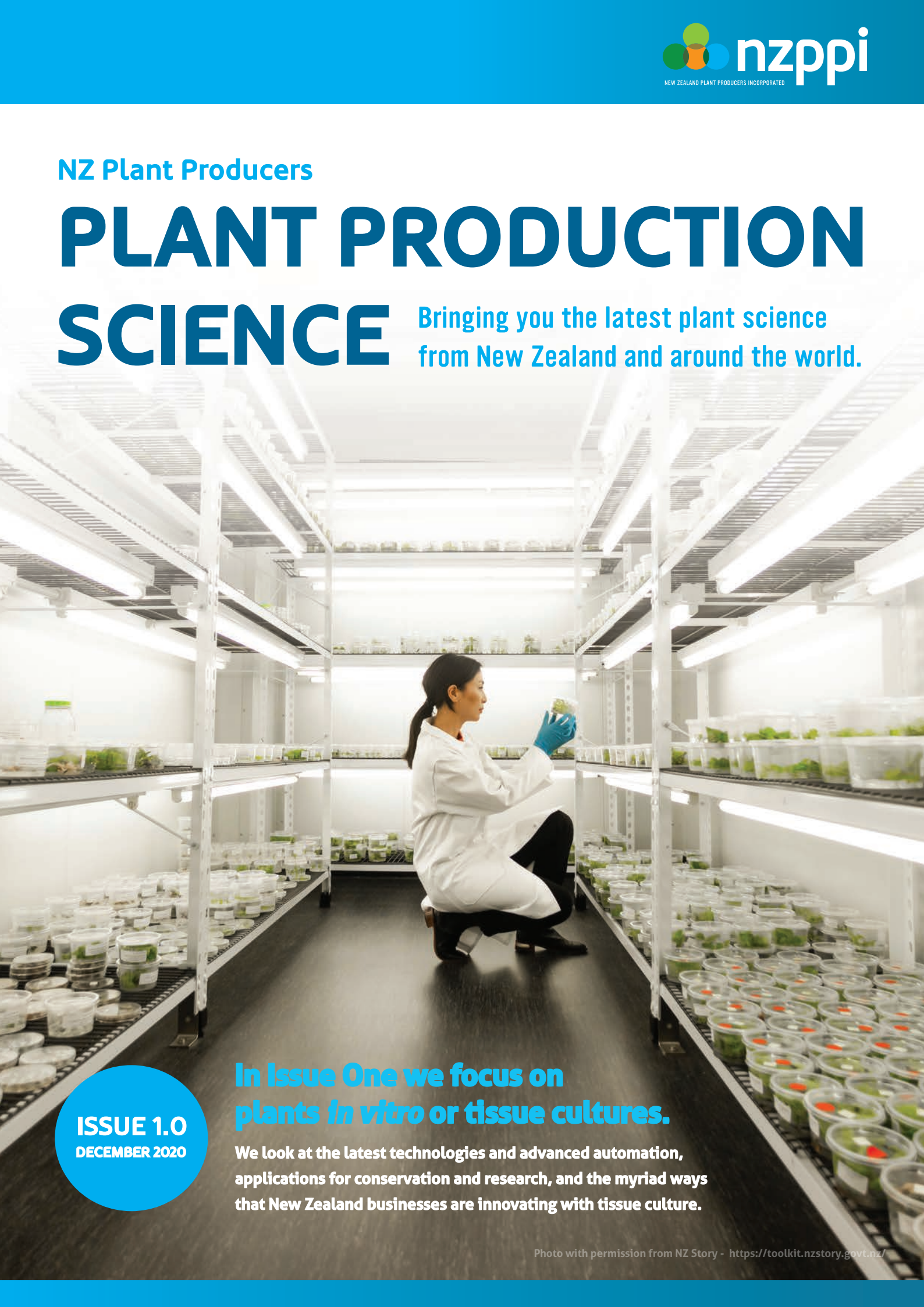

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In Issue One we focus on plants *in vitro* or tissue cultures.

We look at the latest technologies and advanced automation, applications for conservation and research, and the myriad ways that New Zealand businesses are innovating with tissue culture.

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Welcome to Plant Production Science, *in this issue we look at how to grow more plants, faster*

Plant tissue culture is changing fast, with new technologies and methods enabling it to be used in new ways to accelerate and control plant production in horticulture, forestry, and conservation and biosecurity management.

Tissue culture propagation is more technically demanding and expensive than cutting or seed propagation, because it's manually intensive and needs specialised controlled environments. But just as automation has revolutionised seed sowing and transplanting, these technologies are being adapted to the tissue culture environment.

Robotics, laser technology, and advances in machine learning are set to revolutionise tissue culture initiation and micropropagation as robots learn how to make precise cuts in plant material *in vitro*. With automated transplanting, these technologies will revolutionise the sector and replace thousands of hours of manual labour, reduce costs and improve efficiency.

In this issue, we look at two companies in Australia which have developed automated systems to change the way tissue culture plantlets are handled.

We also look at new applications for seed embryogenesis in pest and disease management as well as improving the rates of seedling germination for native plants.

And in the International Year of Plant Health 2020, we look for ways we can reduce the risk of pests and diseases in imported plants.

INTERNATIONAL YEAR OF PLANT HEALTH 2020

Kathryn Hurr
Biosecurity & Technical manager
NZ Plant Producers

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Lifetech Laboratories – global connection

Photo: Mark and Jimmy inspecting tissue cultures.

Lifetech Laboratories Limited commenced business in Auckland in 1981 and was one of the first tissue culture laboratories in the world.

Originally set up to propagate plants for the New Zealand cut flower industry it soon developed to produce hundreds of thousands of plants per year for the US and European nursery markets, including supplying the original breeders of those plants. The business still exports the majority of its tissue culture production today.

In addition to the micropropagation laboratory, Lifetech also operates a Level 2 and Level 3 post-entry quarantine tissue culture facility, as well as having a greenhouse for plant trials and holding mother plants. The business' R&D function completes plant science project work across a number of sectors including food crops, forestry, hops, etc. Lifetech is licensed by several large US and European breeding companies to import elite stock material which is then used for tissue culture production. The business is trusted to hold and maintain a genebank for overseas breeders and plant producers. Lifetech is also a global 'Route to Market' distributor of plant varieties for several New Zealand breeders.

Mark Wilson bought into the business in 2014 and says that New Zealand's high biosecurity reputation – as a country, our government regulations, and good biosecurity business practices – make us a trusted plant producer and exporter in the eyes of our overseas markets. Biosecurity rules also have a big impact on the ability to get material into the country – so rules need to be based on sound science and good risk assessment. The New Zealand domestic market is probably only 20% of the size of the Australian market, so it's an advantage if our rules are broadly aligned to Australia.

The laboratory currently employs 20 staff – some of whom have been with Lifetech since the 1990's. New Zealand's labour rates are more expensive compared to overseas, so Lifetech targets on optimising their growing protocols (light cycles, temperature control, subculture lengths, media recipes) to make them as effective and efficient as possible. The business is focused on growing plants which are recognised by others as harder to produce, such as variegated plants that require experienced technicians to judge whether they are stable, and will have the exact variegation specifications that makes those plants sought after.

Since Covid-19, the availability of international airfreight space has dropped dramatically and has meant Lifetech has had to work hard to get its tissue culture plantlets to customers. Recently they have received an increasing number of enquiries from New Zealand businesses looking to initiate tissue cultures from plants and cultivars which are already in the country.

In August next year, Lifetech will be 40. It could be the time to get out the calculator and work out how many 100's of millions of tissue cultures plantlets have been produced by this New Zealand plant propagation business.

Mark Wilson
Managing Director – Lifetech Laboratories Ltd.

Waimea Laboratories Ltd
– a new venture for Waimea Group

Waimea Group in Nelson has purchased the Plant Propagation Laboratories Ltd tissue culture production facility from owner Peter Bennet. The new business will be named Waimea Plant Laboratories Ltd.

Photo: Production manager for Waimea Nurseries Dave Harris, right, checks on tissue culture plants with PPL owner Peter Bennet.

Waimea Group director Bruno Simpson said the opportunity to purchase PPL comes at a time when Waimea Nurseries requires increased quantity of propagated apple rootstocks to meet growing demands in horticulture and the garden industry. Waimea has made several visits to the USA and looked at techniques to speed up production for new stool-bed production.

One of the focuses for WPL will be introducing Geneva® series rootstocks into tissue culture production allowing the rapid increase in production quantities. It will also introduce new apple rootstocks to help the industry with improved productivity and pest and disease management.

It can take five to 10 years to get commercial volumes of new plants using traditional techniques. Tissue culture production offers a quicker way of bulking up new apple rootstocks, taking half the time to produce commercial volumes. It also presents an opportunity to try other methods of propagation for varieties that have been difficult to do using traditional methods.

Waimea Plant Laboratories is pleased to confirm the business will remain in Hawke's Bay, with all 16 staff retained and Bennet staying in the business for another 18 months. WPL will continue to produce the current product range for the existing customer base of nurseries and retailers. *"We are excited to continue the business that Peter has built up over the last 30 years and to now expand the lab's product range into other species and cultivars,"* Bruno said.

The new company will extend its product range to include blueberries, strawberries and hops and will invest in the modernising laboratory facilities, improving and digitising business systems and continuing to build and develop a great team, with the goal to become New Zealand's leading tissue culture laboratory in the coming years. Waimea is currently investigating the Nic-In tray system for automated transplanting to improve efficiency.

A new acclimation facility will be built in Nelson within 12 months to improve the overall success rate of the hardening off process based on similar systems used offshore. This will sit alongside the company's recently completed propagation facility that was designed to support growing on significant volumes of tissue culture plants.

Bruno Simpson
Waimea Group

Innovation in tissue culture automation

Photo: Nic-In Systems Automated transplanter

Tissue culture production is a time-consuming and manually intensive process. The need for sterile technique in each stage is paramount. Automation is now available that will revolutionise the transplanting process.

I spoke to Nico Van Rooijen of Nic-In Systems® about the technology he developed for his plant production business in Australia. Nico looked around the world to find automated transplantation systems for tissue culture and found a big gap in the market. He set about adapting the technology used by other industries to the tissue culture environment.

While the initial goal was to improve production efficiency, many other improvements have been realised. Most tissue cultures around the world are produced in round "takeaway" containers. By switching to rectangular containers, 30% more plants can be grown in the same space. The growing room is an expensive space to power lights and keep temperatures and humidity within an acceptable range, so extra plant production makes a big difference.

The Nic-In system has also led to improvements in transportation of plants. The agar growing medium is very fragile and if the container gets knocked during transit, or has any pressure applied, the agar breaks up and turns into a kind of soup. Bruised plants can fail their border inspection and get thrown out. The cells in the Nic-In system have a smaller volume of agar and holes in the bottom of the cell trays mean that agar can flow from one cell to another in the boxed tray. With freight costs up 400% and the massive reduction in air-freight post-Covid times, it costs as much to ship tissue culture from Australia into NZ as it does to ship into the USA. Reducing plant losses saves cost.

Photo: Plants growing in Nic-In tray system.

Photo: Individual plants in agar cell plugs.

The Nic-In trays are a 7 by 7 cell tray, containing 49 plants. Many people like the system because it saves having to manually count plantlets! Improvements in robotics has meant the transplanting systems used for tissue cultures can handle the plantlets in almost the same way that seeds get handled. Two splathes are inserted into the cell and lift the plant with the plug of agar up, the robotic arm moves sideways and plants the agar plugs into their final pots. Different transplanting heads are available, and different agar mixes are used depending on the species. There is a lot less transplanting shock as the root systems remain completely intact and are precision planted at exactly the right depth every time. A single transplanter can do 14,000 - 15,000 plants per hour and do the same work that would take 30 people per day, so this is a significant savings in labour costs. Smaller transplanters can plant up to 5000 plants per hour.

Major developments are just around the corner which will enable the micro-propagation process to be automated. The equipment uses laser technologies and machine learning to train computers exactly where to make precision cuts (even zig-zag cuts!) and with no risk of contamination.

Nic-In was poised to sell systems into several very large plant breeding and cut flower companies in Europe but Covid-19 put paid to those plans earlier this year. Now the dust has settled and six months later it has become even more apparent that one of the greatest risks to many businesses is a guaranteed labour force. Automation is now seen as a necessity for business continuance.

Nico van Rooijen
Majestic Young Plants / Nic-In Systems
Victoria, Australia

Total automation for high health clonal plant propagation

Photo: Lowes TC+ ®Bioreactor.

Greg Lowe of Lowes TC Pty Ltd in Australia has developed another type of automated system based on a completely different technology.

"It is basically a 'hydroponics in the lab' system using all of the sterile techniques and controlled light and temperature you would expect in a tissue culture environment. Liquid media moves up into the plant growing chamber for a few minutes every eight hours. The plants therefore remain in a dry environment so that they can grow normally," Greg says. The liquid system reduces the need to transfer plantlets from one medium to another, to produce shoots first and then roots.

The TC+® Bioreactors have been in use for 2 years now and have been found to produce up to 100% increase in the yield of plants and a 30-40% decrease in labour costs, across many different plant species. With automation, Australian tissue culture laboratories are able to compete on cost against imported 'unrooted cuttings' from low labour cost countries, where the airfreight and quarantine inspection costs can be higher than the plant cost. Greg estimates that every plant that comes out of TC production in Australia has between 30 to 50 cents worth of labour in it. *"Every time we touch the bioreactor, we are moving 150 or 400 plants, so we are not touching them one at a time. We touch them in mass, and we produce them in mass,"* Greg says. Hundreds of thousands of plants have now been produced from the system and Greg is confident it works commercially.

"We can also start to harden the plant off inside the bioreactor by increasing the air flow. The cuticles start to harden, and the stomata start to work. They are still softer when they come out of the bioreactor than a normal plant out of a greenhouse. However, they are much hardier than normal tissue cultures". This saves on nursery deflasking [hardening] costs as well, so the labour cost of a fully hardened plant ready to pot is less than AUS$0.01 cent per plant. It can be used with existing pick and place greenhouse automation equipment to size up the plants into the final cell size for sale.

Photo: Dianella plants in the Bioreactor growth chamber

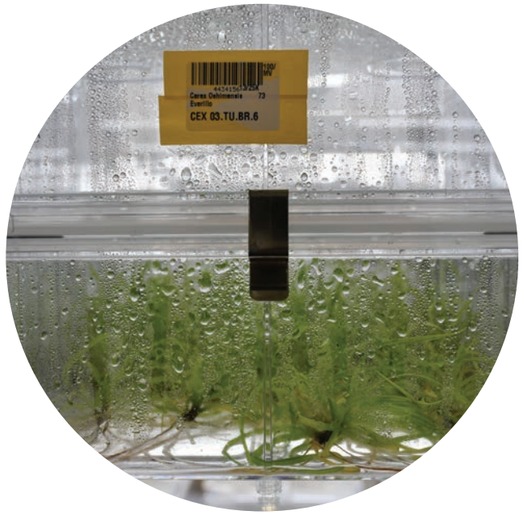


Photo: Carex in the Bioreactor growth chamber

They have also developed a 'game-changing' automated propagation system - the AX® automation - which is still under trial and under wraps. It uses a simple machine [no robots, Artificial intelligence machine learning or vision systems!] to cut each of the 250 plants in the TC+® Bioreactor at the same time. The machine cuts each plant into 2 to 6 uniform pieces with a single action, taking just 2-3 minutes for each bioreactor. Using multiple bioreactors, the process can produce 10,000 to 30,000 plants per hour if there is enough starting material to keep up with the machine. These rates mean the labour cost to produce each plant is well under AUS$0.01 cent per plant.

Greg says these combined technologies mean they can now outcompete imported tissue culture plants on price and allow them to meet the needs of the Australian markets with high-health clonal material for growers, farmers and producers.

The first fully automated plant tissue culture propagation machine is being set up within a clean room at their lab near Sydney which they hope to demonstrate to the world in 2021. They are currently in collaboration with 8 horticultural industries in Australia, including the sugar cane industry, several other food crops, as well as pharmaceutical, forestry and regeneration crops. It is exciting times at Lowes TC but they want to prove the system completely before they offer it for sale or license to other companies.

Greg Lowe
Director, Research & Development
Lowes TC Pty Ltd., Australia

Tissue culture for conservation

Photo: Jenny Aitken with a Kahikatea tree at Owhango

Dr Jenny Aitken has worked with many different tree species in tissue culture, including Californian redwoods and New Zealand kauri. I spoke to her about the potential myriad of applications for tissue culture in the forestry and conservation estate.

When people think of tissue cultures they tend to think of it as a cloning technology, producing thousands of genetically identical plants. There are certainly lots of applications in the forestry sector producing trees with certain desirable traits, and investigations are underway to produce clones that are resistant to diseases in the natural environment, e.g. canker-resistant Cypress clones. Such techniques might also be applied to produce myrtle-rust resistant clones of *Lophomyrtus*, for example, should plants with this trait be found in wild populations.

Where genetic biodiversity is the aim, non-clonal tissue culture techniques may be more suitable. The huge surge in interest in restoration planting of natives and alternatives to radiata pine has created demand for some species which are in short supply. Most New Zealand native species are best sown using fresh seed and even then, sometimes the rate of germination is very poor, or erratic germination occurs over several seasons. Where the seed is old, germination rates are often very low.

'Embryo rescue' is a method where the seed embryo is carefully excised from the seed and germinated on nutrient agar medium. It is being investigated for several New Zealand species and may offer the ability to propagate plants more quickly and achieve greater success rates than might otherwise be possible using seed germination. This could ensure greater numbers of more difficult-to-propagate species are available for replanting programmes in the future.

Embryo rescue may also offer an improved importation pathway for plant species, as the embryo is usually sterile and free from any contamination which might be transported on or under the seed coat.

Jenny Aitken
The Tree Lab

Scion tackles NZ wilding conifers

Photo: Seedlings in the nursery

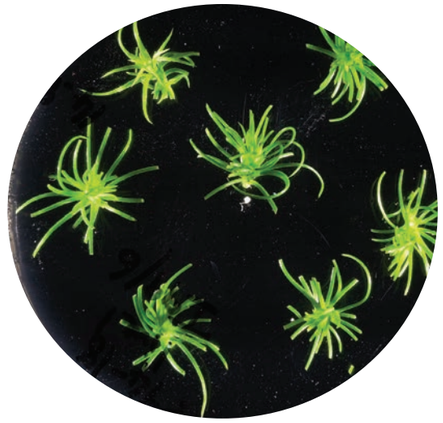


Photo: Douglas fir in tissue culture

Scion has developed a somatic embryogenesis protocol to multiply up shy-coning douglas-fir trees as a novel solution for wilding conifers.

Douglas-fir (*Pseudotsuga menziesii*) is an important forestry species and contributes $200 million to New Zealand's gross domestic product. It is also a wilding conifer species that costs the country more than $11 million each year to control in the landscape.

Finding trees that consistently produce small numbers of cones with few seeds offers a potential solution to the problem. So-called 'shy-coning' trees, have been identified in Ernslaw One forestry production seed orchards north of Gore. Scion researchers are keen to investigate whether seedlings produced from these trees also produce fewer seeds, which could reduce the spread of volunteer trees in wilding-prone areas. However, producing thousands of new trees from a small number of cones and seeds is challenging using normal seed propagation methods, and douglas-fir doesn't grow well from cuttings.

Scion is using somatic embryogenesis to bulk up young seedlings from the shy-coning trees. The technique uses immature seed embryos from green cones which are excised and then induced to produce masses of cells, each with the potential to produce many copies of the tree. These are given growth regulators to form mature somatic embryos, which can either be germinated to produce roots or deep frozen to revive later.

Once the TC seedlings are hardened off they can be introduced into nursery conditions, ready for field testing. The next step is to see whether the progeny inherit this shy-coning ability, and if this is successful, it will represent a step-change in breeding to improve wilding management of douglas-fir in the natural environment.

Heidi Dungey
Science Leader – Forestry Genetics, Scion

Tissue culture helps protect Intellectual Property

Photo: The world's first raspberry Delphinium

New Zealand developed delphiniums are a world-wide hit – so popular that overseas breeders are taking advantage of locally-developed intellectual property and using it in their own breeding programmes.

New Zealand Delphiniums owner Katrina Hindmarsh is using pollen-sterile cultivars produced via tissue culture as an alternative to seedlines, to help protect intellectual property.

Katrina bought New Zealand Delphiniums (formerly Dowdeswell's Delphiniums) three years ago and has continued to build on the genetic selections developed by Terry Dowdeswell. New Zealand Delphiniums' genetics are sought after in northern hemisphere markets, particularly dwarf pot delphiniums which were developed for the lucrative European and USA markets where potted plants are sold in flower. Katrina has continued with Terry's breeding and selection work and is in the process of introducing a world first raspberry-coloured pot delphinium, double-flowered varieties and other pot delphiniums.

The business had been primarily focused on seed exports to Russia, Europe and the USA but in the past few years Katrina has been trialling the development of one-off plants and has sent trial series to the Netherlands for putting into clonal tissue culture propagation. Many of Terry Dowdeswell's early pot delphinium genetics were not protected by Plant Variety Rights, and Katrina is now seeing Dowdeswell's genetics turning up in overseas breeding work, slightly different but obviously "essentially derived" from the New Zealand genetics. Katrina previously worked as a lawyer, so has a clear perspective on the role of intellectual property rights and enforceability.

Photo: New Zealand Delphinium's in the Whanganui nursery

Photo: A double-flowered Delphinium, yet to be named

Tissue culture with Delphiniums has proven difficult to date. They can be successfully initiated *in vitro* but it appears that 'rooting' is a sticking point and they fade and die. Up until recently, there were only one or two successfully produced TC delphiniums around the world but the number of labs that are achieving success is steadily growing. Delphiniums can sometimes be either pollen sterile or self-sterile. These plants are ideal for clonal TC production as they can't then be used in any further breeding programme by competitor breeders.

Tissue culture is much more economical than clonal cutting production. From a breeder's perspective, it is easier to produce a 'one off' plant than a seedline, as there are so many additional factors which go into producing a successful seedline (parent compatibility, production efficiency (good pollen and seed production capacity of both parent plants); seed vigour and seed storage capacity of the cross. A TC produced plant does not have any of the variability that a seedline produced plant will have, so plants are uniform and arguably easier to protect. From a grower's perspective, the uniformity of the plants mean they are more standardised and efficient to produce.

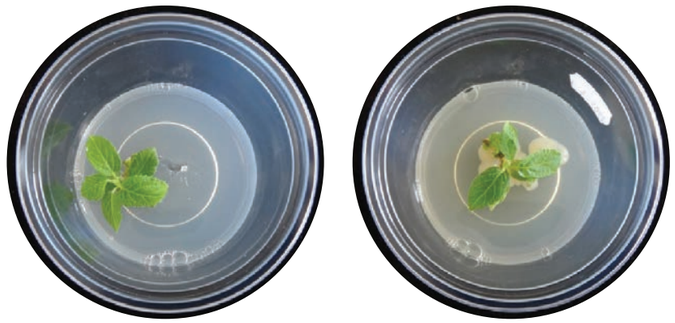
Katrina works with a Dutch agent who grows the trial material in Europe and decides if they want to put them into tissue culture. The agent takes care of all the overseas PVR applications, as there are different processes in Europe and the USA. Once the plants are successfully initiated into tissue culture they are multiplied up and sent to the young plants grower, who grows them to a plug stage, sells them to a liner grower, who then sells them to the plant finisher. The plants thus enter the market having gone through a number of specialist growers.

Katrina Hindmarsh
New Zealand Delphiniums
(Dowdeswell's Delphiniums)

Tissue cultures and plant health

Propagating and distributing disease-free plants is a key element of a thriving horticultural industry.

We are all constantly vigilant for diseases in our nurseries and greenhouses. There is abundant evidence that distributing infected plant material is a significant risk pathway for disease spread in horticultural crops. For example, *Pseudomonas syringae* (Psa) spread is associated with movement of bud wood.



Photos: Tissue-cultured kiwifruit plants were inoculated at the base with Psa bacteria at c. 103 cfu/mL. Two days after inoculation half the plantlets were transferred to tissue-culture medium supplemented with peptone. Left: standard TC medium. Right: peptone-amended TC medium with bacteria obvious as a white growth around base of plant. Photographs taken 16 d after inoculation.

Tissue culture presents lower biosecurity risk than other forms of plant propagation, though the actual magnitude of risk is less well understood. Many people assume tissue culture plants are sterile when in reality, plants have their own microflora in the same way that humans do. Some of the microorganisms carried by tissue cultured plants might be pathogenic, but we also need to consider that some of these organisms may have not yet understood important roles in plant growth and development. Advances in DNA technologies have greatly enabled our general science knowledge in this area and we can expect to see new developments that will improve our understanding of how these may contribute to plant wellbeing.

We do know that some plant microorganisms are true pathogens and they can be maintained in tissue culture across multiple culture cycles and years. We have demonstrated this in tissue-cultured kiwifruit with Psa, where tissue-culture initiated from plants from the greenhouse has suddenly shown signs of infection months or years later.

The Psa bacterium does not grow on standard plant tissue culture medium and contamination is not easily detectable. Following inoculation of tissue cultured plants with Psa, it was only a matter of days before the bacterium was found throughout the entire tissue cultured plant yet there were no visible symptoms.

Peptone encourages growth of a range of bacteria that might be present in cultured plant material, so it is useful for identifying the presence of bacteria. When peptone was added to the culture medium, the Psa bacteria rapidly multiply and can be detected visually.

Ed Morgan
Science Group Leader
Plant & Food Research

Big plans for Plant Health and Environment Laboratory

The Ministry for Primary Industries' Plant Health and Environment Laboratory level 3 tissue culture facility holds imported plants *in vitro* - waiting to be de-flasked and grown in a greenhouse environment for post-entry quarantine (PEQ) diagnostic testing.

It contrasts with other MPI accredited tissue culture facilities, which offer bulking services through tissue culture multiplication while in quarantine.

Besides holding imported plants *in vitro*, the Plant Health and Environment Laboratory can also hold *in vitro* local plants, indicator plants for surveillance - and incursion responses, and PEQ plants where disease testing is required. PHEL can also hold exotic viruses and viroids in infected, tissue-cultured host plants for diagnostics and research purposes, including new organisms in the Rosaceae family.

In the future, we want to explore other opportunities for New Zealand's horticultural industries. The demands differ between each sector. However, one common requirement is for clean plant materials – which could be addressed when the Laboratory expands its service capabilities. There is potential for a purpose-built tissue culture facility capable of eliminating disease from high-value crops. This technology would also enable the international transfer of disease-free plant germplasm from local sources – vital for the future of plant biosecurity and conservation.

There is opportunity to provide micrograft, embryo and shoot-tip culture services combined with either heat and cold therapy. These techniques will eliminate potentially destructive plant pathogens, especially viruses, viroids and phytoplasmas. The ability to eliminate tricky diseases means higher crop yields, reduced pest management costs, better biosecurity, and greater export opportunities and reputation for New Zealand. However, these tissue culture activities require highly specialised skills. We need to upskill our staff or recruit people with the right expertise.

Tissue culture technologies present a great opportunity for MPI and the New Zealand horticulture industry. Technological development of this scale would require a large investment and therefore, is still an aspirational and long-term goal. With industry stakeholder support, MPI could deliver disease elimination services and potentially position New Zealand as a provider of clean plant material.

Sathish Puthigae
Team Manager – Post Entry Quarantine & Botany
MPI Plant Health & Environment Laboratory

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