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NZ Plant Producers

PLANT PRODUCTION SCIENCE

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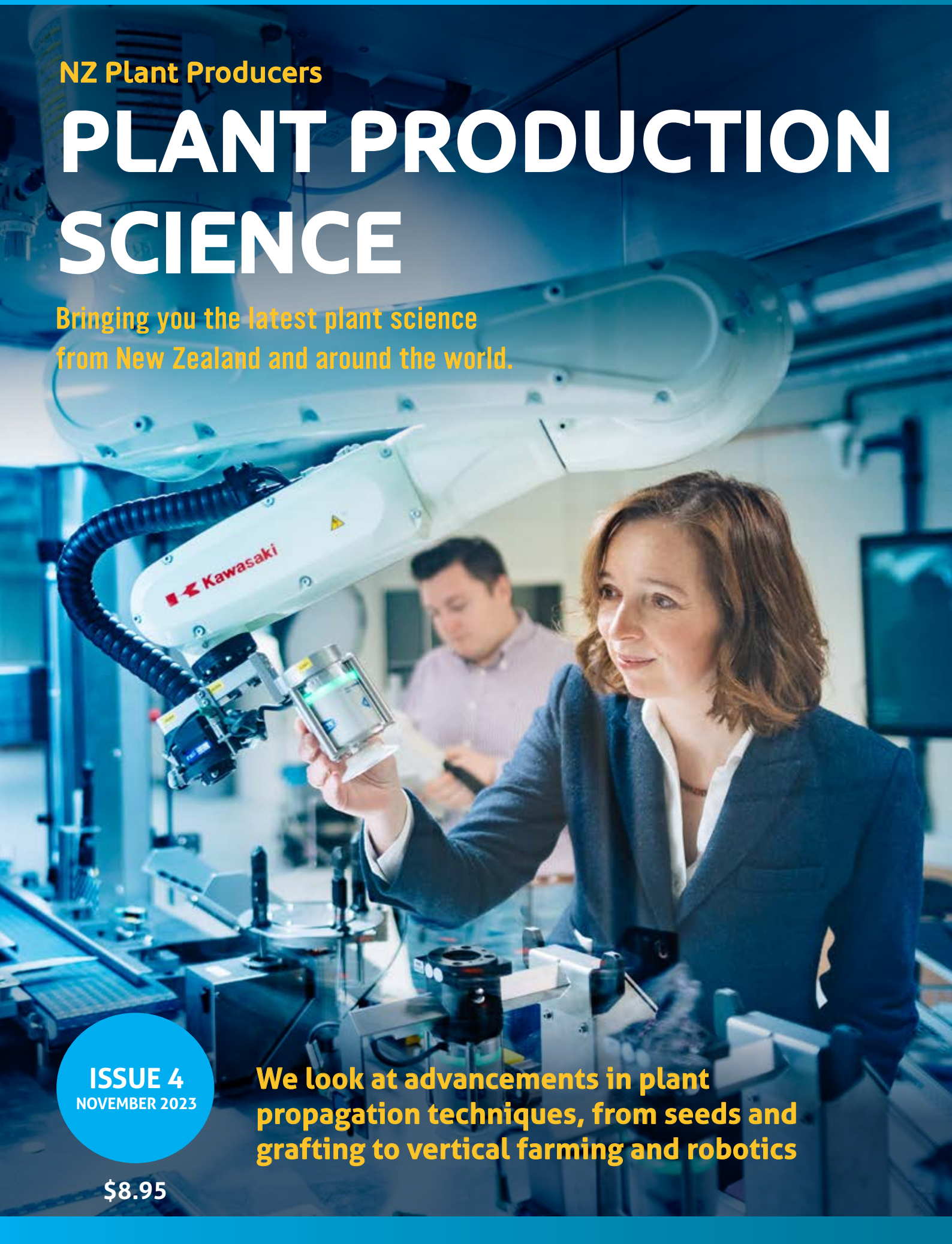

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We look at advancements in plant propagation techniques, from seeds and grafting to vertical farming and robotics

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Contents

Welcome to the fourth edition of Plant Production Science.

In this issue, our expert authors showcase the latest research and advancements shaping plant propagation today. Each article contributes to a broader understanding of the traditional and innovative propagation techniques.

We look at the art and science of grafting, covering topics from innovative hot-callus methods to specialised avocado grafting. We also discuss the nuances of budding, a classic yet evolving method in plant propagation.

We explore vegetative propagation methods with a focus on the propagation of our native trees. Complexities of seed dormancy and germination are investigated and we delve into the fascinating world of fern propagation and mycorrhizal relationships in plant production.

A big thank you to our experts and our advertisers for making this publication possible. We hope you find it a useful resource for your plant production business.

Warm regards,

Kathryn Hurr,
NZPPI Biosecurity and Technical Manager.

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Using science and innovation in your plant production?
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Revolutionising plant production: tissue culture smart tech

Plant production is on the brink of a revolution with the advent of tissue culture smart technology. By integrating robotics, AI, and advanced computing, this innovation promises to enhance in vitro plant growth with precision and efficiency.

Automated tissue culture production is the holy grail of *in vitro* plant production and with recent advances in robotics, coupled with powerful computing and AI capabilities, a prototype will be available in the marketplace as soon as mid-2024.

The journey towards automated tissue culture has been led by Friederike and Stephan von Rundstedt of RoBoTec PTC, a spin-off of Bock Bio Science in Germany. Collaborating with a growing team of experienced plant specialists and software experts, they have dedicated the past 12 years to developing this ground-breaking technology.

Stephan and Friederike von Rundstedt of RoBoTec PTC.

Plant production is on the brink of a revolution with the advent of tissue culture smart technology.

The central challenges have been replacing human intervention with robotics i.e. requiring robots capable of physically manipulating containers and plantlets; developing imaging software that generates a comprehensive 360° view of the plant; and creating sophisticated machine intelligence to determine precise cut-lines using lasers.

Over the years, the team has faced numerous obstacles and encountered failures during the testing and development phases. The initial prototypes aimed to automate specific elements of the process, progressively harnessing the potential of the Internet of Things and advancements in vision technology. The technology was patented in 2014/15 and has received worldwide approval, garnering recognition in the form of the Taspo "Innovative Product" award in 2018, and the Bremen Environment Prize in 2019. The project's total investment has reached €20 million to date.

To bring the first model to market, Bock Bio Science has entered into a partnership with German machine builder Focke/Hoyer. The interest in this technology

extends worldwide, with strategic cooperation from Ball Horticulture Co. in the USA.

The advantages of smart technology in tissue culture automation are evident. Manual cutting with a scalpel can introduce impurities and cause more damage to plant cells compared to laser-cutting, which significantly reduces stress and minimises opportunities for infection. The AI component of the system is well-informed, identifying optimal cutting lines based on the natural growth patterns of each plant, thanks to the knowledge gained from tens of thousands of "perfect" decisions made during prior training. Additionally, the RoBo®Cut system operates efficiently 24 hours a day, ensuring continuous production capabilities.

The development of this smart technology represents a significant milestone in vitro production. The integration of robotics, advanced imaging, and machine intelligence promises to revolutionise the tissue culture industry, increasing productivity and ensuring healthier plant development. With the expected market release in the near future, this innovative technology holds great promise for the future of plant production.

Laser cut vs Manual cut.

Stephan von Rundstedt
RoBoTec PTC, Germany.

Skyward seedlings: vertical farming for reforestation

Vertical farming is revolutionising how we cultivate plants, and its latest foray into forestry could be a game-changer.

Vertical farming technology has been used to grow traditional leafy greens, herbs and microgreens. Successful trials in Scotland have now shown its potential for forestry, with each vertical tower having the potential to grow up to 2.7 million tree seedlings per year.

Kenny Hay from Forestry and Land Scotland (FLS) and Caroline Craggs from Intelligent Growth Solutions (IGS) completed the research trials at the IGS Crop Research Centre in Dundee. They found vertical farm towers can achieve growth rates in tree seedlings six times faster than field-grown trees.

Seedlings established in the field typically take 18 months to reach a plantable size (50mm for conifers and 200 – 500mm for broadleaved species). Conifer species trialled with the vertical farm towers included Norway and Sitka spruce, Douglas fir, Lodge pole pine and Scots pine and performed well. But the real standout were the deciduous tree species... plantable grade silver birch, aspen, rowan, alder and hazel trees were grown in just 90 days, with oaks taking slightly longer at 110 days.

The towers improved germination rates through precise inputs and create a pest and pathogen free growing environment. Each 9m tower system is modular and can be easily scaled up. The 6.4m^2 growing trays are automated and fully customisable, with adjustable (narrow-band) LED lighting, in-situ/flood hydroponic technology, temperature and humidity control, all centrally monitored and controlled. The flexibility allows for highly specific 'recipes' of inputs to be developed depending on crop species and end-product.

With fully adjustable environmental control, the hardening off process can also be started within the tower before moving trees outdoors into the nursery or planting in the field. Any combination is possible, integrating both new technology and existing field or glasshouse systems for plant production. Customising the light parameters can also achieve desired characteristics for planting. For example, slowing down growth rates in some species leads to thicker root collars and sturdier plants for planting.

Kenny Hay with alder seedlings.

Scotland has ambitious goals for planting forests to meet its climate change and biodiversity targets, with targets to establish 25 million new trees annually, planting 12,000 ha across the UK. The vertical tower system has demonstrated the potential to reliably produce uniform trees with better water and nutrient efficiency compared to traditional systems.

This vertical farming breakthrough heralds a new era in reforestation, promising a future where forests can flourish from towers to terrains with unprecedented efficiency and speed.

Kenny Hay, Forestry and Land Scotland (FLS)
Caroline Craggs, IGS.

New plants for a new world

A three-year project at Plant & Food Research is exploring whether inter-species grafting technologies could one day allow any imaginable combination of fruit and plant.

New Plants for a New World programme leader, Dr Falk Kalamorz, says inter-species grafting between plants is currently limited to compatible grafting groups and a limited number of plant tissues.

"Grafts tend to be more successful if the plant species are more closely related, but nobody has completely established what leads to a successful graft".

The development of a compatible graft typically involves three major events: adhesion of the rootstock and scion, proliferation of callus cells at the graft interface or callus bridge, and the differentiation of the vascular cambium across the graft interface.

"Our focus has been on grafts between so far incompatible plant tissues. To get a better understanding of this we've been comparing the structural and molecular events associated with successful and unsuccessful grafts," says Falk.

Grafting (in)compatibility has been the subject of a range of current studies, and several enzymes, structures and mechanisms have been found to play different roles in the process. Nevertheless, our understanding of grafting remains incomplete.

When callus cells come into contact, the cell walls undergo dissolution and new structures like plasmodesmata are formed.

Recent studies have found that plasmodesmata play an important role in cell recognition and cell communication involved in graft formation, and there can be prominent differences between graft partners.

Dr. Falk Kalamorz and Dr Margaret Carpenter (Plant & Food Research) inspect grafted capsicum and tomato plants.

Complex biochemical and structural processes and signals are also involved. Phytohormones, especially auxins, regulate key events in graft union formation, while other hormones, proteins, miRNA and small RNA are important in controlling stock and scion communication.

As part of the project, Plant & Food Research scientist Dr Margaret Carpenter has been overseeing trials involving tomato and capsicum plants, which are known to be partially compatible. She's also conducted trials using pear scions on an apple rootstock, a combination that is currently unviable commercially, as it's known to survive for a few years.

It's hoped more understanding of the structural and molecular events associated with grafting could also improve the rate of success for grafts of already compatible plants, which would unlock significant advances for nurseries supplying orchardists in Aotearoa New Zealand.

"Nurseries in Aotearoa that we've engaged with about this project are really supportive because they know first-hand about all about the challenges associated with grafting, but also the huge potential it has," says Falk.

This experimental grafting research fits within Plant & Food Research's *Hua Ki Te Ao – Horticulture Goes Urban* research strategy, which is focused on developing new plants and growing systems in urban settings, to help meet the need of future consumers. To guide this effort, the team also has social scientists engaging with end-users and consumers.

"In the future, soil conditions may not be suited to some fruit trees, but these varieties could be grafted onto a plant with strong roots that are more tolerant to extreme conditions. By exploring these experimental grafting techniques there's the opportunity to help futureproof food production here and overseas."

Dr. Falk Kalamorz,
Plant & Food Research.

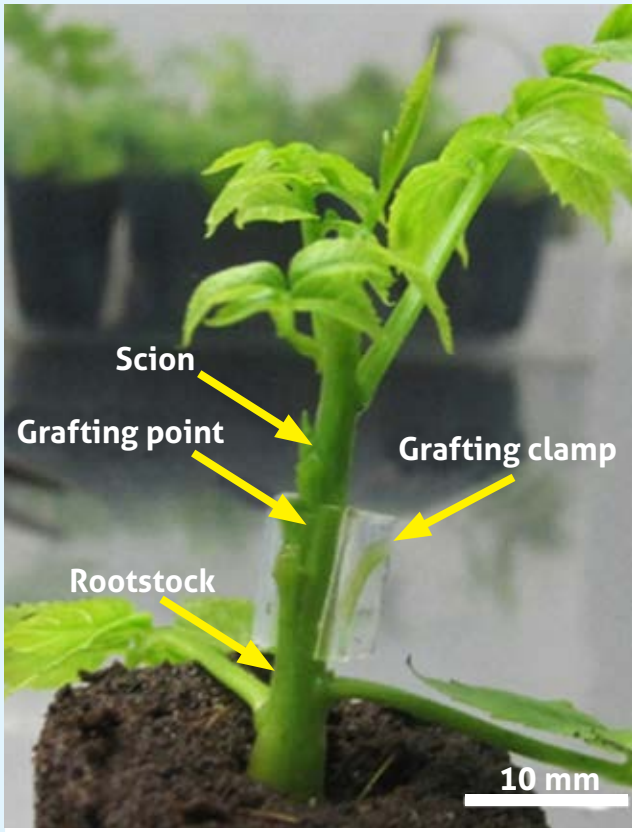
Grafting pre-treatments

Wounding of plant material during scion/rootstock preparation can cause browning and oxidation of plant tissues, resulting in poor graft success.

New research has found that pre-soaking scions in antioxidant solutions such as 0.01% ascorbic acid and 0.015% citric acid (1:1) prior to in vitro grafting helped reduce oxidation. In micrografting of roses, a quick dip treatment in silver nitrate (50 mg L−1) was found to be an effective antioxidant, preventing production of phenolic compounds that could lead to micrografting failure.

However, some studies have found the opposite. Micrografts of *Protea cynaroides* treated with antioxidant solutions induced more browning in scions than in those that were untreated, and the study concluded that the fast operation of micrografting is more important in preventing tissue browning than pre-treatment. The response of antioxidants to the reduction/inhibition of phenolic browning may be species-dependent, and their concentrations and/or combinations are critical points to understand.

The use of nylon microtubes or clamps to hold the scion and stock together has been shown to enhance the success rate of both in vitro grafting and ex vitro grafted plant growth.



Walnut micrografting showing the use of a grafting clamp, from Ribeiro *et al*, 2022.

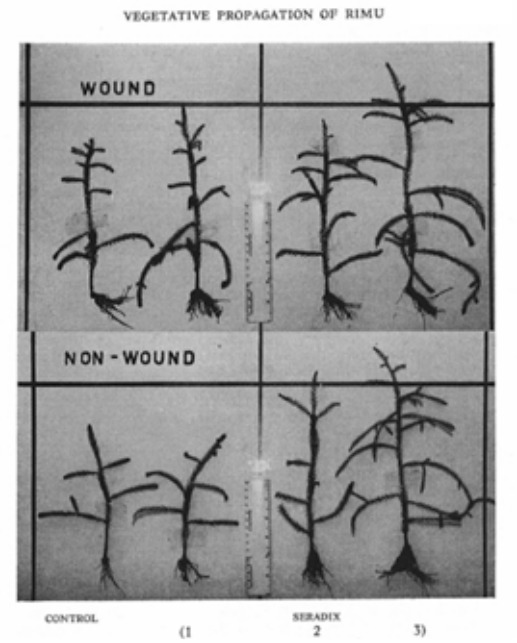
Branching out: innovations in native tree propagation

Propagation of native plants, especially podocarps, can be challenging due to inconsistent seed production and low germination rates. Researchers have explored alternative propagation methods. This article looks at Scion's recent trials and older research in this area.

Seed production in many New Zealand native plants is highly variable between seasons due to marked periodicity in fruiting. There can be periods of two to four years with very little seed production, and much of it is sterile. Percentage germination of species such as rimu (*Dacrydium cupressinum*) can also be low.

Scion recently published their trial work using mini-hedge cuttings techniques on a range of native species.

Seedlings were spaced in sandbeds at 10cm x 15cm and established for two weeks prior to removing apical tips. Shoots were harvested and set into 4cm paper-pots and placed in a rooting tunnel on heated beds. This process was repeated monthly for four months. *Podocarpus totara* produced a high number of harvestable cuttings from the mini-hedges (37 per hedge before setting) with an 80% rooting success. Good results were also achieved for Pohutukawa (*Metrosideros excelsa*). Other species were less successful. Previous trials in the 1970's showed good results for rimu, totara and black beech.



Data from the Daikon study, 1974.

The propagation process for rimu was as follows:

- Cuttings were taken from young nursery stock plants (2 ½ years) grown under shade, taking 10 – 15cm terminal shoots just above a lateral, with a basal cut made at about a 30° angle.
- Cuttings were treated with a commercially available hormone powder and set in a scoria/sand/peat medium and rooted under intermittent mist controlled by an electronic leaf, on a well-drained bench. The hormone powder Seradix 3 (0.8% indolebutryric acid) gave the best results.
- Cuttings set in November showed superiority in rooting over cuttings set in January.
- Wounding of the cutting base did not increase root production in cuttings set in November but appeared beneficial for January set cuttings. Lateral cuttings do not achieve an upright form, perhaps as rimu often exhibits a marked weeping habit even in young plants.

For totara:

- Totara was propagated from semi-hardwood cuttings taken in early to mid-April
- Cuttings were treated with semi-hardwood hormone before leaving them under mist spray in a greenhouse.

For black beech:

- In *Nothofagus solandri*, October gave the best results.
- Spring/early summer period may be the best time to set cuttings, when temperature and day length are increasing, and when stock plants commence active growth.

Other work noted that high air temperatures tend to promote shoot development in advance of root development, suggesting daytime air temperatures between 21 – 27°C, with a night temperature of 15°C would be beneficial, with a uniform temperature near 21°C at the cutting base. Bottom heat may be of little advantage during the warmer months but may be useful for initiating root development earlier in the season.

Vegetative propagation of New Zealand native podocarps, like rimu and totara, appears promising as an alternative to seed propagation. Techniques involving cuttings, hormone treatments, and strategic timing have shown success, overcoming challenges like seed unavailability and inconsistent quality, and enhancing nursery production viability of these native species.

Rotorua to host 2024 international conference on advancing vegetative propagation technologies of trees

The international IUFRO (International Union of Forest Research Organizations) 2.09.02 conference on tree vegetative reproduction is being hosted by Rotorua and Crown Research Institute, Scion, in March next year.

IUFRO is a globally recognised non-profit, non-governmental, and non-discriminatory network for voluntary cooperation of forest research organizations in various fields (9 scientific Divisions). It is inviting researchers from all backgrounds to submit their work on advances and application of vegetative propagation of trees and other woody plants. PhD students are especially encouraged and can receive all-expenses-paid trips.

The conference, titled "The might of vegetative propagation for healthy and productive forests to face climate challenges," will bring together global researchers, professors, PhD students, and forest stakeholders to collaborate, share knowledge, and tackle critical environmental and socio-economic challenges for sustainable forestry.

The conference will explore various aspects of vegetative propagation, from conserving and enhancing tree genetic resources to deploying adapted varieties for resilient and productive forests. Field trips are planned, including visits to clonal forest and NZPPI member, Minginui Nursery, where cutting-edge propagation technology has been used to restore land and create community employment opportunities.

Details:

IUFRO 2.09.02: The might of vegetative propagation for healthy and productive forests to face climate challenges

Novotel Rotorua Lakeside, Rotorua, New Zealand 3-8 March 2024

For further information on IUFRO 2.09.02 and to register, visit the official conference website: **https://iufro2024conference.wordpress.com**

Spore to splendour: mastering fern propagation

In the world of ferns, propagating these ancient plants from spores is both an art and a science. Paul Michael, a seasoned fern cultivator, unlocks the secrets of nurturing these spores using meticulous process, honed over twenty years.

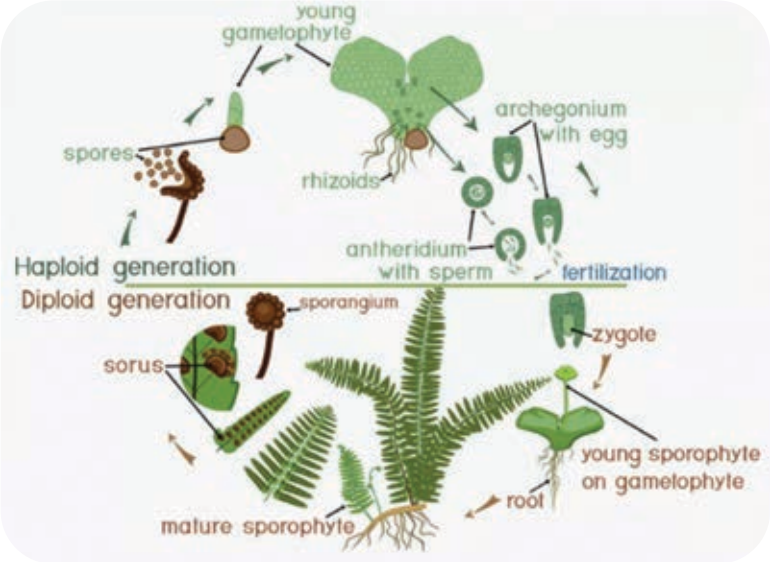
Paul Michael owns Fern Factor in Christchurch and has been propagating ferns from spores for the past 20 years. He has a purpose-built laboratory with stainless steel benches and a laminar flow hood to prepare growing media and measure out the spores.

Some ferns can be propagated by division or offsets (bulblets) but most species are propagated in larger numbers using spores.

Ferns undergo an alternation of generations to complete their life cycle. In the first stage, a spore will germinate and grow into a prothallus (singular) or prothalli (plural), which looks like a green scale. The prothallus is the **gametophyte**, or sexual stage of a fern's life cycle.

The prothallus produces round craters, which hold the egg and sperm. Fern sperm are motile and can swim in water to fertilise eggs on other prothalli. Once fertilised, the egg and sperm grow into the second phase of the fern's life, the non-sexual **sporophyte** stage which grows into the fern with leaves that produce spores.

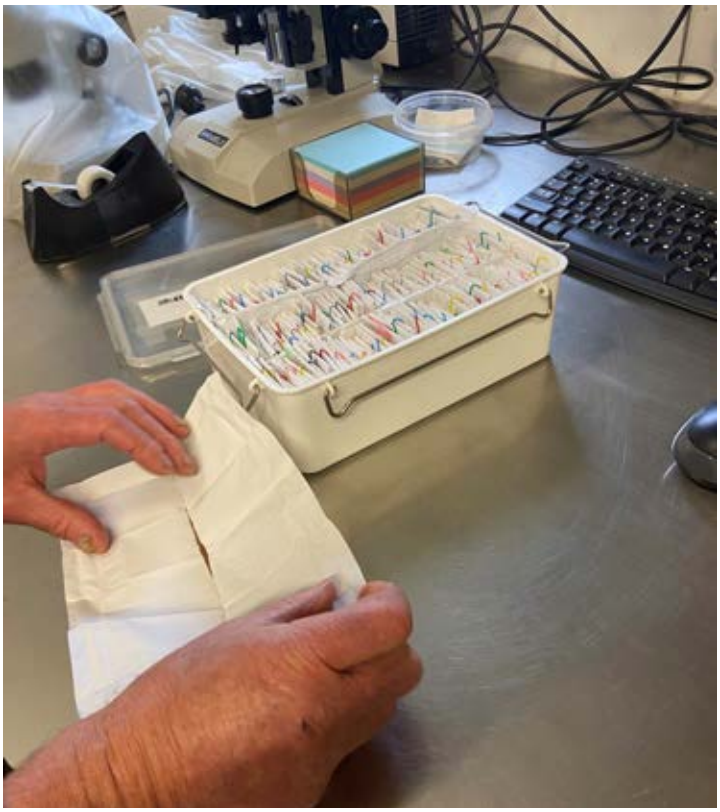
Alternation of Generations



Fern alternate generations as part of their life cycle.

Paul's main principles for germinating ferns from spores:

1. Collect fern fronds or pinnae with ripe sori, which will look plump and firm with little brown, black, yellow or green spheres. Spent sori are generally a dull cinnamon brown. Cleanse the frond by briefly soaking or swishing in a 5 to 10 percent bleach solution to cut down on foreign contaminants. Rinse with running water, shake and place the fronds, sporangia side down on clean paper.
2. Separate the spores from the debris by carefully lifting and gently tapping the paper from underneath, coaxing the debris to slide off the paper and discard it. Spores can also be sifted through tissue paper used for cleaning camera lenses. Pack spores in smooth paper envelopes ready for use.



Paul Michael of Fern Factor.

3. Cleanliness is the crucial. The propagation medium and the container must be as sterile as possible. Sterilise all equipment, and tools using a disinfectant like bleach or alcohol. Moistened sowing medium (mixture of peat moss and perlite or vermiculite) can be sterilised in the oven or microwave. It should reach a temperature of 80°C for 30 minutes to prevent contamination and growth of mould, mosses and liverworts.
4. Once the mix has cooled, sprinkle the spores thinly and evenly on the surface. Avoid overcrowding to give enough space for the developing gametophytes.
5. Fern spores require a consistently moist environment for successful germination. Cover the container with a clear plastic lid or plastic wrap to create a humid environment. Fern spores generally prefer diffuse light during germination and most prefer temperatures between 20°C to 25°C for successful germination.
6. It can take several weeks to several months for the spores to germinate and develop into young fern plants. Once the gametophytes have developed into young fern plants they can be carefully transplanted into individual pots and gradually acclimatised to increased light and reduced humidity to help them adapt and grow stronger.

Paul Michael,
Fern Factor.

Unlocking early harvests: the science of avocado grafting

In the quest for quicker, abundant avocado harvests, grafting has emerged as a pivotal technique.

All commercially grown avocados are grafted in the seedling stage to shorten the juvenile phase, leading to earlier fruit production compared with trees grown from seeds.

Avocados were traditionally grown on a seedling rootstock where a fruiting scion was grafted onto a germinated avocado seed. Most commercial growers now plant trees grown on clonally propagated rootstocks, limiting the genetic variability that can be seen with seedling rootstock and ensuring the genetics of the rootstock are identical to the superior mother trees.

Clonally propagated avocado trees ready to be severed from the mother seed. Note the rootstock graft just above the seed, and the fruiting scion graft at the top of the plant.

Producing avocado trees on clonal rootstocks requires grafting to be done in two steps.

First, a rootstock selected for environmental or disease tolerance is grafted onto a germinated seed using a cleft graft. Once the graft union has formed and the rootstock buds begin to grow, the plant is transferred into an etiolation room to grow in the dark. After approximately three weeks, the plants are taken out of the etiolation room for processing. Processing involves wounding a small part of the shoot stem, applying rooting hormone and then placing a small pot with growing media around this area to encourage root growth. Once roots are initiated and growing, a fruiting scion is then grafted onto the rootstock, selected from mature, high-yielding trees with fruit of desired quality and characteristics. Once the fruiting scion is growing well, this micro-clone plant is severed from the seed and planted out into a larger bag to grow on ready for planting.

The whole process takes 12 to 18 months from seed germination to having a tree ready for commercial planting.

Grafted tree maturity is governed largely by the scion. Recent investigations into the molecular mechanisms of tree maturity have found that avocado seedlings and rootstocks have a juvenile microRNA (miRNA) profile compared to budwood from mature trees (see text box). Avocado seedlings have a high abundance of miR156 while budwood material have a lower abundance, and vice versa for the downstream targets *PaSPL4* and miR172. Avocado clonal rootstocks (grafted from mature trees) also have a similar miRNA profile to the budwood material, retaining the maturity level of their source trees. These molecular studies confirm a graft transmissible regulation of genes which have a central role in regulating floral development and juvenile-to-adult phase transition.

Additionally, recent studies in avocado have shown evidence for graft transmissible regulation of genes, involving leaves below the graft union. Retaining a few leaves on the grafted rootstock could help with molecular signalling between the grafted scion, leading to a more successful graft. Other evergreen may also benefit from this approach.

The exploration of grafting avocados reveals a sophisticated interplay between traditional horticultural practices and cutting-edge molecular insights, offering a glimpse into the future of enhanced tree maturation and the potential for broader horticultural applications.

MicroRNAs (miRNAs) are a class of non-coding RNAs that play important roles in regulating gene expression. miRNAs have now been linked to most aspects of plant biology. They were first identified in plants less than 20 years ago, but they have been shown to be critical regulators of developmental process such as leaf morphogenesis, vegetative phase change, flowering time, and response to environmental cues.

Sarah Williamson,
Lynwood Avocado Nursery.

Transforming plant cultivation with automatic grafting technology

Grafting is an age-old horticultural technique that has been around for millennia, dating to ancient Greece and 2000 B.C. China. However, the technique has changed little throughout this time. The process of splicing and combining scions and rootstocks (for woody crops) is just as labour intensive as it was 4000 years ago.

Viscon Plant Technology in the Netherlands is working to develop an automated and robust grafting machine for woody crops. They are looking for producers of fruit, citrus, nut trees and other woody crops to get involved in the development process.

When needed, the machine can have interchangeable blade and positioning setups to accommodate different grafting techniques.

The grafting process comprises three steps;

- Precisely cut both scion and rootstock
- Accurately position them towards each other
- Carefully seal the graft with tape or plastic clips

Some grafting techniques can be automated more feasibly than others. Splice (whip) and cleft grafting are the most accessible, but also possibilities arise for wedge, saddle and whip & tongue grafting. Below, a compiled list of grafting techniques and the fruits they are considered best practice for.

Grafting technique	Fruits
Splice (Whip) Graft:	*Apple, Pear, Persimmon, (Japanese) Maple.*
Cleft Graft:	*Apricot, Grape, Orange, Mandarin, Lemon, Lime, Grapefruit, Pummelo, Coffee.*
Wedge Graft:	*Lychee, Avocado, Fig, Mango, Macadamia, Eucalyptus, Cocoa.*
Whip & Tongue Graft:	*Plum, Damson plum, Walnut.*
Bark (Side Veneer) Graft:	*Sapodilla, Pecan, Cashew.*

Get in touch with Viscon Plant Technology if you are interested in participating in the development phase of this grafting innovation: viscongroup.eu/news/development-of-automated-grafting-machine-woody-crops/.

Warm unions: hot-callus grafting

In the complex world of tree cultivation, hot callus grafting emerges as a crucial tool for propagation success. This technique, a blend of precision and controlled warmth, has altered the way we foster the growth of challenging tree species.

Many tree species are difficult to propagate from hardwood cuttings, traditional field budding and grafting techniques. Hot callus grafting can significantly increase the chances of grafting success.

It has been used internationally on various fruit crops and tree species such as *Acer, Corylus, Fagus, Juglans* and *Quercus*.

Mitchell Patching and Amber Elmslie of the Manawatu have been using the hot callus grafting technique for the past two years to propagate select cultivars of *Betula populifera* and *Liriodendron tulipifera*.

Based on techniques developed and patented by Lagerstedt in 1981, the hot-pipe callusing system provides localised heating of graft union to about 26°C, while keeping the rootstock and scion wood cool (about 7°C) to prevent their premature growth. The elevated temperatures at the graft union help to accelerate cell division, resulting in the formation of new callus tissue and callus bridge formation between scion and rootstock.

The technique is performed in the late winter or early spring while the rootstock and scion are dormant. Rootstocks are carefully selected so that the scion and rootstock diameters match at the graft union. To reduce contamination, all grafting knives, tools, and the bark of

Betula Whitespire, one of the species being produced with hot-callus grafting.

Heating cable is laid inside a chamber to provide warmth to the graft unions.

Close up of hot tube.

scions and rootstocks are cleaned with an alcohol based cleaner before any cuts are made.

The grafting is a whip and tongue graft, using very sharp knives to make a really clean cut and graft union is secured using grafting tape.

Harrisons Trees use a 65mm Marley PVC downpipe (round profile) as the heating chamber. The heating cable is suspended inside the pipe without touching the graft. 10mm slots are cut into the plastic tube to enable the graft union to sit inside the pipe. The entire system is insulated around the graft with insulating material and the scion and roots are kept cool.

The *Betula* grafts were ready in 4-5 weeks using this process, as assessed by the formation of callus.

Referring to various plant species, including walnut, Lagerstedt (1984) suggested experimentation of at least one year to adapt the hot callus grafting technique to the local climate, facilities, work schedule and plant material.

Important aspects for success:

- Optimal time of grafting
- Method of heat supply to the graft union
- Duration of the hot callus period
- Different cultivar responses to hot callus conditions
- Effects of rootstock genotype

Mitchell Patching,
Harrisons Trees Ltd.

The art of budding

Budding is a form of grafting in which a bud is inserted into the bark of a compatible rootstock to create a beneficial variety and rootstock combination. It is the best propagation method if the propagating material is scarce and valuable. Budding unions are stronger than grafting and is comparably simpler and quicker.

To successfully bud, the scion and rootstock must be compatible, the scion buds must be fully developed and dormant, and ultimately the meristematic tissue from the scion and rootstock must be aligned with good contact. Special care to prevent the buds from drying out is necessary to complete the process.

Budding on table.

Josh Brooks and Michelle Booth budding *Ulmus lutescens* onto elm rootstock in late summer.

Chip budding and T-budding are the most common types of budding for fruit crops and woody ornamentals. The type of budding method used is dependent on the bark's slipping (an indication of the rate of cambium/healing activity), compatibility of the stock and scion, coinciding with the period of active growth in the season. Among the different methods, chip budding is more versatile as it can be used when the bark is not slipping freely.

Selection of bud-wood

Select vegetative buds, which are usually small and pointed. If transporting bud wood from another place, remove leaves while leaving the petiole intact and pack the bundle of bud-wood in moist jute, cloth or sphagnum moss. Budwood on some species can be too fresh and splitting is an indication of this. In these cases, leave foliage on the fresh budwood until it is slightly limp, or prepare the budwood one day ahead of requirement and store dry sealed in a bag in the chiller.

Time of budding

Budding time usually depends on the availability of well-developed budwood, which can occur at different times of the year depending on species.

Spring budding:

Commonly done in *Citrus*, the period of spring budding is short and should be completed before the rootstock has made much new growth. The bud must be dormant, and the rootstock is ready as soon as the bark slips easily on the rootstock.

The rootstock top is cut on a slant after 2-3 weeks from budding, once healing has taken place.

Deciduous species may be spring budded using wood collected in July and chip budded in early October. Middle and upper buds on the rod are best for this method as all are matured and will produce stronger plants.

Summer budding:

This method is preferred in areas with a relatively long growing season and is mostly used for stonefruit. Budding is done in the early part of the growing season. A seedling of 30cm height, 3-5mm diameter can be used for budding. The bud is taken from the current season's growth and is forced to sprout immediately. Usually, 3-4 leaves are retained below the bud.

Healing takes 2-3 weeks, and the rootstock is immediately cut, leaving at least one leaf above the bud and several below. A good indication of bud union is the petiole dropping off cleanly, if it is strongly adhered and starts shrivelling and darkening, it indicates budding failure. Other sprouts should be rubbed off to ensure fast growth of the scion bud.

Autumn budding:

Autumn budding is done in late summer on tropical, subtropical, and temperate fruit and nut species, especially in areas where the growing season is short. The rootstock is large enough by late summer to accommodate the bud, the plants are actively growing and the bud bark slips easily. As bud sticks are selected, leaves should be cut off immediately, leaving only a short piece of leaf petiole to help in handling the bud. The best buds on each stick are usually on the middle and basal portion, as the succulent terminal portion is usually immature.

After the buds have been inserted and tied, the plants are left until the following spring until the buds start growing. Tension of the ties is important for budding at all times to hold the bud firmly in place and as the season progresses this becomes critical as the healing slows down at the end of the season.

Citrus chip bud begins to sprout 10 days after budding.

Citrus 7 weeks later the stock is ready for removal.

Types of budding

The main budding methods and a short description

Shield or T-Budding & Inverted T-Budding	The most common, used on plants with thin bark. A 'T' shaped cut is made in the rootstock bark 15-20cm above ground level, with the two flaps then loosened with the budding knife. A shallow cut is made about 5-6mm below and 2-3cm above a healthy bud which is inserted into the "T" and tied with a polyethylene strip. Inverted T-Budding is the same as T-Budding except the horizontal cut is made on the bottom of the incision and the bud inserted up into the separated flaps. This method is effective due to the downward flow of plant hormones intercepted below the bud, making for a faster healing process and stronger bud union. It also prevents the possible entry of water, so is used in areas of high rainfall.
Patch-Budding	A rectangular patch of bark is removed completely from the rootstock and replaced with a patch of bark of the same size containing a bud. Widely used in thick-barked species. The thickness of stock and scion should be of the same size. The patch should be wrapped and hold the bark tightly, covering all cut surfaces to prevent entry of air, water or pathogens.
Ring or Angular Budding & Flute Budding	Used in peach and mulberry. A complete ring of bark is removed from the stock to girdle it. A similar ring of bark containing a bud if inserted onto the rootstock. The thickness of stock and scion should be of the same size. If the bud fails to heal in, the stock above the ring may eventually die. Flute budding is similar, except the bark is not completely girdled and a narrow band of bark is left. A similar sized piece of bark with a healthy bud is inserted into the vacant area and wrapped tightly. The stock thus remains alive even if the bud fails to sprout.
Chip Budding	A chip of bark and wood is removed from the stock and replaced with a chip of similar size from the bud wood. The cambiums should be lined up, then the chip tightly wrapped, leaving the bud uncovered.
I-Budding	Most appropriate where the bark of the rootstock is much thicker than that of the bud stick. The bark of rootstock is cut using two transverse cuts and then a single vertical cut or produce an "I" shaped cut. The two flaps of bark are lifted out and a bud patch inserted beneath them and tied, ensuring the bud patch does not buckle outward and leave a space between the rootstock.
Forkert Budding	The stock receives two vertical cuts and a transverse cut midway to create a flap, with the lower portion of bark removed. The scion is similar to a patch bud and is slipped into the exposed portion of the stock and the flap drawn down before tying.
Micro budding	Used in Citrus, only the bud is utilised. The petiole is cut off just above the bud and a flap cut is made just under the bud. The tiny shield containing the bud is inserted into an inverted "T" cut on the stock and then tied.
Top budding	The 12cm diameter and insert 1-3 buds on the upper side of the remaining limbs using the T-Bud method. Remove unwanted buds and sprouts to ensure only the desired scion buds grow.

Vance shares some of his comprehensive hints from years' experience with grafting and budding.

Cuts	Timing / Sap flow	Polarity (growth direction)	Humidity (preserve scion health)	Post graft training
	DECIDUOUS GRAFTS			
Whip & tongue 1/3,1/3,1/3	June – August to September stored wood	Topgrafts first choice Polarity mostly not important, Ginkgoes possible, *Cornus contraversa* definitely	Tying and sealing cuts	De-suckering, trimming and staking
CRAFT KNIFE factory edge	Black tape like spot welding >5°C warmer			Pre-graft removal of rootstock eyes, auxins from growing tips suppress other buds
All cuts slicing motion				
Machine grafts crunch cut				
	EVERGREEN GRAFTS			
Cleft graft	Mostly August fir spring flush	Top of stock >> auto dominance	Tent for humidity bagging grafts	Remove rootstock shoots but maintain foliage on stock to feed roots.
Black wrap to union will improve results as in deciduous grafts.	Chill and dry conifers. Graft dormant > warmth	Scion selection esp. conifers, polarity of scions critical. Side graft > harder to force dominance.	Remove leaves on some species. Leafless wrap of scions.	
	BUDDING			
Budding knife	Wood matured to firm, and more dormant than rootstock	Occasionally shows in growth. *Cornus contraversa*	Overhead water often fatal for container buds	Heading back in early spring will reduce suckering to some degree. Too late in spring, sap flow will interfere with sealing the cut stocks.
All cuts slicing motion				
Strop, occasional stoning				
	Rootstock must be active		Summer buds and rain	
Allow for bark thickness of different species	Spring budding done with well-developed buds. *Cornus* veneer grafts			De-suckering before suckers shade and compete with the growing buds.

Vance Hooper,
Magnolia Grove.

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Unlocking the secrets of seed dormancy

Seed dormancy is nature's way of timing plant emergence with optimal environmental conditions. It's a survival mechanism that ensures seeds only germinate when their chances of thriving are highest. This article by Jayanthi Nadarajan of Plant & Food Research looks at the various classes of seed dormancy and the intricate environmental cues and treatments that can trigger germination.

Seed dormancy is a physiological state that inhibits or prevents germination, even in favourable conditions. Whilst a non-dormant seed can germinate across a wide range of favourable conditions, dormant seeds can only germinate when the dormancy is broken or alleviated.

Seed dormancy mediates the interaction between the growing environment and seedling establishment. Dormancy prevents seeds germinating during periods favourable for a short period, such as a warm spell in mid-winter.
This interaction takes place through various physiological, morphological, and anatomical mechanisms or signalling within the seed in response to the environment.

In general, seed dormancy has been divided into five main classes:

- physiological dormancy (PD),
- morphological dormancy (MD),
- morphophysiological dormancy (MPD),
- physical dormancy (PY) and
- combinational dormancy (PY + PD).

Physiological dormancy is the most common and responds to seasonal cues to ensure that germination only occurs after specific environmental events. The response of PD seeds to the growing environment is highly specific, and germination can be triggered in response to a particular set of temperature, chemical, or light signals. PD can be a major contributing factor in a plant's life-trait expression, e.g., by determining if temperate species behave as winter annuals or summer annuals. Temperature is one of the main factors

that influence the depth of PD. In some non-tropical species, dormancy can be alleviated in hydrated seeds after a period of low temperature exposure during winter. This method of relieving dormancy is known as moist cold stratification and usually carried out in the temperature range of 0 – 10°C. It is commonly practised in forestry and horticulture. On the other hand, some species may require moist warm (≥ 15°C) stratification for their germination. The length of exposure to low or warm temperature is also critical and growers should take note of the species' natural growing habitat when germinating seeds of species with which they are not familiar.

Fluctuations in light and temperature can also terminate dormancy. In the natural habitat the magnitude of daily temperature fluctuations will be sensed by seeds with the size of these fluctuations depending on their depth of burial in soil. Seeds buried near the soil surface experience a wider range of temperature fluctuation compared to deeper buried seeds, producing a stronger signal to terminate dormancy.

Morphological dormancy (MD) is associated with seeds that are dispersed with embryos that are undeveloped or partly grown and need to grow before they can germinate. Extending the postharvest period allows the embryo to grow inside the seed prior to germination. Though MD can be influenced by environmental conditions, in comparison to PD, these types of seeds are less flexible in adjusting dormancy behaviour to environmental conditions. Plant hormones, in particular abscisic acid (ABA) and gibberellins (GA), mediate plant developmental processes and regulate whether the seed remains dormant or germinates. ABA is known for inhibiting germination whereas GA is known to supress ABA and promote seed germination. Seeds with undeveloped embryos can also have physiological dormancy (PD). In general, if embryo growth and radicle emergence are completed in about 30 days under suitable conditions, the seeds are considered to pose only MD. If germination takes more than 30 days, and seeds require additional dormancy-breaking treatments such as stratification and GA hormone treatment to germinate, they are known to have the combination morphophysiological dormancy (MPD).

Some seeds have impermeable seed coats that prevent entry of water and oxygen, causing physical dormancy (PY). The presence of a cuticle layer, suberin, lignin or cutin all contribute to hard seed coats. In natural habitats, PY can be broken by temperature fluctuations from freezing and melting of soil water, soil micro-organism activities, forest fires or animal digestion. Seed PY can be overcome by mechanical scarification (such as rubbing seeds between pieces of sandpaper, or with severe shaking, or sudden changes in temperature such as a brief dip in boiling water, or piercing the seed coat using a needle) or by chemical scarification (using sulfuric acid, enzymes such as cellulase and pectinase or organic solvents such as alcohol and acetone). Embryo rescue using tissue culture is also applicable to overcome PY.

Understanding and managing seed dormancy aligns horticultural practices with the natural tempo of the environment, ensuring that seeds awaken to germinate at the peak of conditions conducive to growth and survival.

Jayanthi Nadarajan,
Plant & Food Research.

Assessing seed excellence: viability, germination, and vigour

High-quality seeds are the cornerstone of successful plant production, leading to robust seedling development and increased yields. This article delves into the critical aspects of seed quality and the advanced testing methods used to assess them.

Use of high-quality of seeds ensures high seedling establishment, increasing crop production and profitability. Seed quality can be measured by seed purity, mechanical damage, seed health (infestation), through physical inspection. Structured laboratory-based tests are required to determine seed viability, germination and vigour.

A viable seed is capable of germination under suitable conditions. While the ultimate method to assess seed vigour is by testing germination, seed viability can be predicted using techniques such as the triphenyl tetrazolium chloride (TTC) test. The TTC test indicates the number of viable seeds in a sample that can produce normal plants under suitable germination conditions, even if the seeds are dormant. This is particularly useful for freshly harvested seeds that possess high levels of dormancy such as observed in some grasses and native species, and for species where optimum seed germination conditions have not been identified.

The TTC is a biochemical test, which differentiates live tissues from dead based on the activity of respiratory enzymes. Upon seed hydration, the activity of dehydrogenase enzymes results in the reduction of the colourless tetrazolium salt solution (2,3,5-triphenyl tetrazolium chloride) into a compound called formazan. Formazan stains living cells (respiring) red while dead cells (not respiring) remain colourless (Figure 1). The advantage of the TTC test is that it takes much less time (24–48 hours) than a germination test which may need several months for completion. This approach must be optimised on a species-by-species basis as results can be affected by the permeability of the seed coat.

The seed coat (testa) may be hydrophobic (water impermeable) because seed coat cell walls contain a range of lipids, suberins and polyphenolics such as lignin and tannins. In such cases the seeds may require chemical or physical scarification to allow water to penetrate or even vacuum infiltration to ensure fast permeation of the TTC staining solution.

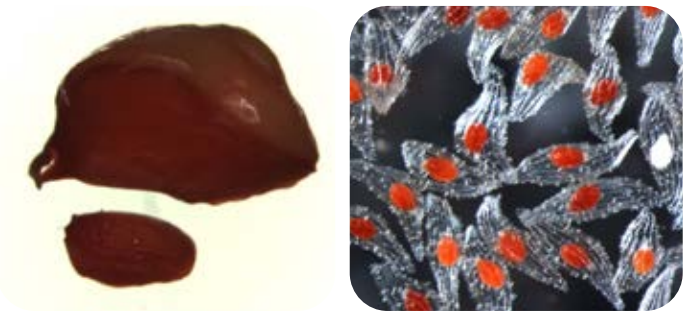


Figure 1. An example of tetrazolium viability test results for (a) viable citrus seed and embryo (a) and orchid seeds showing viable embryos stained red (b).

Seed vigour is defined as the potential for a seed to complete the germination process and establish a healthy normal seedling. Seed germination conditions need to be optimised based on the species' natural habitats and origins. In general, temperature and photoperiod regimes are the two main factors affecting seed germination. As a rule of thumb, species from tropical regions would require high (25–35°C) and constant germination temperature with a 12 h photoperiod. In contrast seed of temperate species would generally require lower temperatures

Figure 2. Seed germination on top of, in rolled and in pleated paper media

(10–20ºC) and usually alternating low/high temperatures with 16 h photoperiod. However, this requirement can be species specific.

Seed germination tests can be carried out on different media like germination papers, sand, soil, perlite, 1% water agar in Petri dishes, etc. When using germination paper as the medium, the seeds can be sown on top of the paper, rolled up in the paper or the paper can be folded to make pleats (Figure 2). The paper medium provides easy evaluation of seed germination but requires constant re-wetting and removal of seedlings to the nursery, and can sometimes be tricky as root hairs penetrate the paper.

Seed germination in sand boxes works well for large seeds (Figure 3). It minimises disease risk as the sand can be heat sterilised before use. Sand medium enables normal root development and easy transfer to nursery media. However, as for the paper medium, sand would need frequent rehydration depending on seed and container size.

Seed germination on water agar minimises dehydration injury as Petri dishes are usually enclosed to minimise evaporation. This medium allows for easy incorporation of dormancy-breaking chemicals. However, water agar medium can be prone to easy spread of infection, and it can be difficult to extract seedlings for nursery planting if they are left too long in the medium (Figure 4).

Germination paper cardboard infused with nutrients is the latest technology developed for seed germination. This method is particularly useful to provide nutrients, dormancy-breaking treatment chemicals and/or seed priming agents. Upon wetting, these agents become active and support the germination and formation of healthy seedlings (Figure 5).

It needs to be emphasised that vigorous seeds are expected to complete the germination process and produce healthy, normal seedlings. It is typical for a proportion of a seed lot to stop at the radicle (root) emergence stage or to produce abnormal seedlings which should be considered when estimating the final numbers of seedlings required for large-scale plantings.

Figure 3. Seed germination in sand box

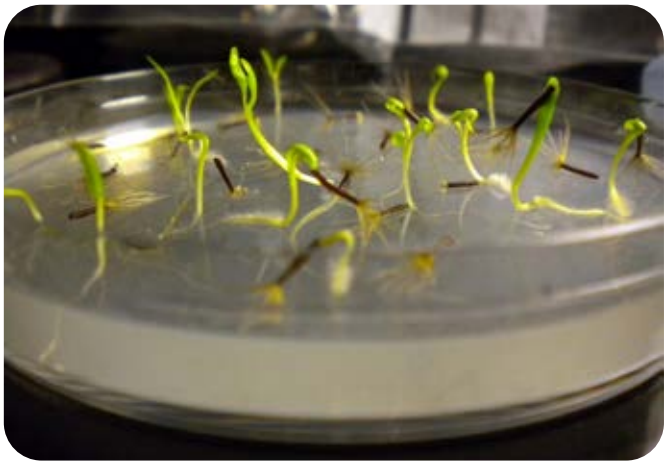


Figure 4. Seed germination on water agar medium

Figure 5. Seed germination on cardboard infused with nutrient and seed priming agents

Jayanthi Nadarajan,
Plant & Food Research.

Grounded in symbiosis: cultivating mycorrhizal giants

Understanding the symbiotic relationship between fungi and plants is crucial for sustainable cultivation. Mycorrhizal trees, with their complex root-fungus networks, play a pivotal role in nutrient uptake and soil health. This article by Dr Alexis Guerin-Laguette of Mycotree in Christchurch looks at the process of producing mycorrhizal trees and the implications for both the environment and industry.

Most land plants form an intimate symbiosis with soil fungi, which colonise their short lateral roots and transform them into mixed plant-fungus organs called "mycorrhizae" (from Greek, "fungus-root"). Fungi are made of thin filaments called hyphae whose diameter is as small as a few µm, i.e., about 100 times smaller than the plants' finest roots!

There are several types of mycorrhizae. Ectomycorrhizae (ECM) form a thick mantle, or fungal sheath, enveloping the colonised root tip. ECM fungal hyphae penetrate the root and grow in between root cortical cells forming a fine network called the Hartig Net, by which plant and fungal cells exchange water, nutrients, and other molecules. Arbuscular mycorrhizal fungi (AMF) grow within the plant cortical roots, forming microscopic tree-like structures within root cells called 'arbuscules'. AMF are much more ancient than ECM and are dominant on ≈70% of plant species from grasses, most crops, shrubs, fruit trees to subtropical and tropical trees, including NZ' Podocarps. In New Zealand, beeches (*Nothofagus* spp) are by far the main native trees forming ECM.

The thin fungal filaments develop like a 3D 'spider web' within the substrate they explore (soil, organic matter), and are able to extract nutrients from minerals, decompose complex molecules, and extend the root systems of plants, considerably enhancing access to water and nutrients that they pass on to their host plant. However, this service for the plant is not free! Mycorrhizal fungi need to source soluble sugars, and fats, and find them... within the roots of living plants! It is estimated that between 2 to 20% of plant-fixed carbon is thus passed on to their mycorrhizal fungal partners.

Mushrooms and Truffles: what are these?

The main body of the fungi that produced them is much bigger and perennial, but invisible, growing beneath our feet in soil and on the roots. Mushrooms and truffles are the fruiting-bodies full of spores, sorts of fungal 'seeds'. Not all mycorrhizal fungi form large fruiting-bodies but most ECM fungi do, explaining why temperate and boreal forests are full of mushrooms, toadstools, and truffles every season. Several ECM fungi form fruiting-bodies that are not only edible but also of great culinary value, i.e., prestigious truffles, porcini, chanterelles, milk caps, Caesar's mushrooms etc. The cultivation of edible mycorrhizal fungi is a very recent activity in human cropping history.

Mycorrhization: How does a tree become mycorrhizal?

In nature, young seedlings germinating near their parent trees become rapidly colonised by the dense mycelium colonising the soil in which they grow, or by soil-dwelling fungal spores whose germination is stimulated by roots growing in their vicinity. In a nursery, mycorrhization is a complex process, only partially understood, its success is not always predictable.

Only a few ECM fungi can be successfully manipulated, in the lab or in the nursery, through the production

In nutrient-poor, natural soil conditions, the effect of the pre-mycorrhization of seedlings by EMF can be very pronounced. Here, the growth of *Pinus massoniana* seedlings after out-planting, pre-mycorrhized (right) or not (left) in the nursery by *Lactarius vividus*, an edible milk cap species native from China. The uninoculated seedling on the left grew slowly at first but is itself naturally colonised by resident soil ECM fungi. For trees like oaks and pines, the association with mycorrhizal fungi is compulsory.

of a fungal inoculum and its application to virgin roots of a receiving plant. The vitality, cleanliness and purity of inocula (fungal spores or vegetative mycelium) are paramount to ensure that the receiving plants will be almost exclusively colonised by the target fungal species.

The inoculum must be applied to the roots of the receiving host plants in specific conditions (potting mix, pH, temperature, moisture etc) and then acclimatised in the nursery. At least one year is required to produce a truffle or mushroom mycorrhizal seedling ready for out planting, and up to 2- or 3-year-old seedlings are commercialised. It is important to test the seedlings after inoculation to ensure they have satisfactory amounts of target mycorrhizae on their root systems. In February 2022, the New Zealand Truffle Association adopted a Quality Standard for edible mycorrhizal fungi seedlings based on state-of-the-art knowledge that specifies and guarantees the quality required of seedlings for establishing EMF orchards. This is a great step forward for the NZ EMF industry. Two nurseries have committed to the standard so far (**https://www.nztruffles.org.nz/where-to-buy-truffle-trees/**) but in time, all conscientious nurseries will do so.

Beyond the culinary and economic benefits of truffles and mushrooms, their sustainable cultivation, appears to be good also for the environment. Mycorrhized trees perform well in the field and together with their underground mycorrhizal root systems, are significant carbon sinks.

Mycorrhizal trees are one of the silent champions of sustainability, anchoring a future where symbiotic strength underpins ecological resilience and prosperity.

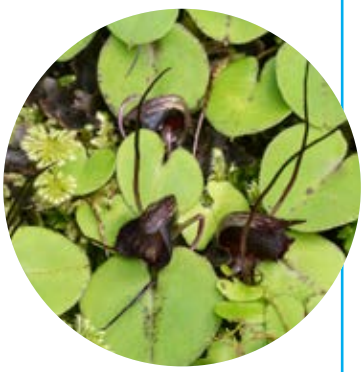
Dr Alexis Guerin-Laguette has more than 25 years of experience as an academic in edible mycorrhizal fungi biology and cultivation. Through Mycotree, Alexis has partnered with NZPPI member, Southern Woods Nursery, to certify their production of truffle inoculated seedlings.

Orchid mycorrhizal fungi

Research indicates that most, if not all, orchid species cannot germinate without the presence of a particular fungal partner. Karin Van der Walt (Conservation and Science Advisor at Wellington City Council's Ōtari Native Botanic Garden) and her two collaborators Jennifer Alderton-Moss (research technician at Ōtari) and Dr Carlos Lehnebach (botany curator at Te Papa) are currently investigating how to propagate some of our threatened native orchids.

Orchid mycorrhizal fungi and the orchid plant don't have a symbiotic relationship. The orchid seed depends on the fungi to germinate in nature, but it is not clear what the fungi gets in return. These mycorrhizal fungi can differ for each orchid species and orchids may form a life-long association with a single fungal partner, while other orchids might require different fungi at various stages of the life cycle.

Karin and the team are applying their research to both common and rare native orchids, including green hoods (*Pterostylis*), spider orchids (*Corybas*) and potato orchids (*Gastrodia*). She collects their seeds and uses the upgraded Lions Ōtari plant conservation laboratory for seed and fungal cryopreservation while she carries out various experiments.



Silver back spider orchids (*Corybas macranthus*)

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