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Forestry Roles for Propagation

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Summary

This paper gives a brief outline of Proseed nursery's main propagation programmes, some New Zealand plantation forest research developments, and thoughts on how modern propagation systems might assist native reforestation.

INTRODUCTION

Proseed is a part of the Ngai Tahu Holdings portfolio. It was originally established in 1966 as the NZ Forest Service Seed Store. As part of Rogernomics dissolution and sale of the NZ Forest Service, in 1987 it was created as a separate business by the NZ Forestry Corporation and was eventually sold to Ngai Tahu in 2001.

Proseed now operates a 161 ha specialist forest seed orchard, nursery, seed production and extraction facility near Amberley. It contracts to the NZ Radiata Pine

Breeding Company to run their breeding archive near Waipara.

Proseed is a member of all major NZ tree breeding programmes: the Radiata Pine Breeding Company, the Specialty Wood Programme (non-durable eucalypts, cypresses and Douglas-fir), and NZ Drylands Forest Innovation.

Proseed sells genetic gain! Seed is the vehicle for that. Around 3 tonne of seed (mostly control and open pollinated, improved *Pinus radiata*) is sold annually. Last season it was 4.7 tonne (equivalent to 94,000 ha of planting)! Proseed is the largest forest seed producer in Australasia.

Proseed nursery programmes include grafting *P. radiata* and eucalypts, propagating pine and eucalypt species from cuttings, plus various incidental industry support projects.

Grafting *Pinus radiata*

Most (110 ha) of Proseed's 161 ha is given to production of control pollinated and open pollinated *P. radiata* seed. Production is from grafted elite selections from the NZ Radiata Pine Breeding programme (**Fig. 1**).

Figure 1. Grafted elite *Pinus radiata* ramets in Proseed's Amberley orchard, bagged ready for controlled pollination.

Proseed completes up to 18,000 grafts annually for orchard establishment and renewal. Planting a recently purchased, neighbouring, 27 ha property will have this continue for a few years yet.

Candle buds are grafted onto regular 1yo container rootstock in winter using a wedge and cleft technique. Grafts are planted out the following winter (**Fig. 2**).

Figure 2. Newly completed *Pinus radiata* grafts. Candle buds are grafted onto one year old seedling rootstock using the wedge and cleft technique.

Grafting Eucalypts

Proseed has established seed orchards with selections out of national improvement programmes for *Eucalyptus nitens*, *E. fastigata*, *E.regnans*, and *E. saligna*.

As a founding member of NZ Dryland Forest Innovation, a group initiative to develop a hardwood industry based on improved durable eucalypt species, Proseed is supporting the groups programmes through establishment of *E. bosistoana*, *E. globoidea*, and *E. quadrangulata* orchards using clonal selections out of respective breeding programmes.

Grafting eucalypts is a summer activity (**Fig. 3**). Freshly collected scions ("ripened" shoots trimmed of all foliage and drenched with a systemic fungicide) are fitted to rapidly grown rootstock (about 16 weeks from sowing) using a cleft graft technique. Grown on in a greenhouse environment, take (and failure…) is rapid: 4 – 6 weeks will show.

Figure 3. *Eucalyptus bosistoana* scions starting to move 6 weeks after completion. Stem sections are grafted onto vigorously growing seedling rootstock (about 16 weeks from sowing) using the wedge and cleft technique.

Some species (*E. nitens*, *E. bosistoana*) are relatively straight forward while others are more difficult. *E. globoidea* is particularly so and a study this summer is investigating efficacy of grafting clones onto their own, open pollinated (half sib) progeny.

Eucalypt Cuttings

Vegetative propagation of elite genotypes from cuttings affords fastest deployment and greatest genetic gain compared to propagation from seed, that is, growing cuttings produces an exact copy of the donor tree. With seedling offspring, the maternal genome is diluted by the pollinating parent and may not come so true.

Thus, parallel to establishing a breeding population and production of improved seed, the NZDFI wished to establish a programme for cutting propagation of early selections from *E. bosistoana* and *E. quadrangulata* base populations. Proseed accepted a mission to root cuttings for establishment of clonal testing trials and for commercial scale planting. Over 6 years NZDFl tasks were:

- Evaluate coppice as a material source.
- Develop a protocol for cutting propagation of *E. bosistoana* and *E. quadrangulata*.
- Produce ramets of 2000 selections to establish clonal testing trials.
- Produce stools (**Figs. 4 and 5**) of 31 juvenile plus trees (~3 years) selected on growth rate, early form, and wood properties, for commercial scale production.
- From those stools, produce 25,000 rooted cuttings for commercial deployment.
- Evaluate strike of cuttings from 10-year-old ortets.
- Report on best practice for propagating *E. bosistoana* from cuttings.

Figure 4. Select *Eucalyptus bosistoana* stools in a crop cover tunnelhouse, used to produce a bulk supply of rooted cuttings for commercial scale planting.

Eucalypt Cuttings for Clonal Trials

Initial work, modelled on what had been seen during a study tour of Narromine Nursery in Australia (owned and managed by David Cliffe, also an IPPS member) was to produce plants for clonal trials.

Cuttings were set into propagation cell trays, filled with loose perlite and peat media, and placed over bottom heat under mist.

From cuttings taken off 2,000 field selections over two seasons, 13,000 plants representing 1,100 clones were produced for clonal trials.

Figure 5. Pilot stool bed establishment with *Eucalyptus bosistoana* (left) and *Pinus x attenuradiata* (right). *E. bosistoana* stools were started as rooted cuttings potted into PB3/4. Once established and growing strongly, some were set up in the hydroponic dripper installation shown here. PB bases were cut away before they were stood on coir growbags for roots to grow through. *P. x attenuradiata* seedlings on the right were grown on in planter bags, potting into larger grades, up to PB8, as growth necessitated.

Eucalypt Cuttings for Commerce

Twenty-one clones for commercial deployment were drawn from the selections that had been propagated for clonal trials. One year into propagating those 21 clones, all breeding values were updated with new additional information from breeding trials. Consequently, nearly all the original set of clones were replaced. That left Proseed nursery just two years to turn about 300 ramets of 31 clones into 25,000 rooted cuttings.

The adopted strategy was first, to produce as many stools as fast and as big as possible in the time available. Most were grown on as regular potted plants in a shade house while a few were put into a hydroponic set up inside a small tunnel house. Growth of hydroponic stools was spectacular, but nutrition was tricky and plants in the shade house produced more robust material.

Early on, bad root distortion in the propagation cell trays was detected. At the time, work setting pine minicuttings into plugs was working well so that same system was successfully applied to the eucalypts.

Nearer to scheduled sales for planting, potting rooted cuttings switched from PBs into forestry tubes to emulate presentation of standard forestry planting stock. In the end, 15,000 plants were produced. Plants were distributed to privately owned sites through the length of New Zealand: North Canterbury to Northland.

In summary, the average strike improved from just 30% at the beginning to 75% at the end. Huge variation between clones, from nothing to 90%, was consistent throughout the programme. Average strike from older (10 year) material was similar, which was encouraging for bringing in older selected trees from breeding trials after completion of more conclusive assessments.

Supporting government funding for this breeding programme has all but dried up. Because viable commercial production of rooted cuttings was not demonstrated, this propagation programme has been suspended, though stools of the clonal set used have been kept.

Propagation Plugs

At the moment there are 2 plug systems in New Zealand to choose from: Ellepot, a paper tube filled with your own media (**Fig. 6**), and Jiffy Preforma plugs, moulded from peat and binding agents (**Fig. 7**).

Proseed evaluation of strikes in each system were similar. Cost is about the same (~$0.10 ea). For Proseed's purpose, the Jiffy system proved more convenient: no special machinery nor messy filling required, preformed dibble holes and no propensity to drop media from the bottom.

Unlike soft stemmed pine cuttings, eucalypt cuttings don't require a dibble hole, so plugs were simply inverted when traying up. (Plugs without a dibble hole are available.)

Conversely, for other nurseries, the ability to produce a range of plug and tube sizes at scale and filled with specialised media would be more advantageous.

Figure 6. *Eucalyptus quadrangulata* cuttings in Ellepots.

Figure 7. *Eucalyptus bosistoana* cuttings in Jiffy Preforma plugs.

Table 1. Pros and cons for plug systems:

System	Pros	Cons
Ellepot	Biodegradable container	Needs specialised filling machinery
	Fill pots with custom media	Dibbling may be required
	Range of pot sizes	May lose media from base
	Specialised tray systems	Paper may turn roots if not wet
	Easy patching and sorting	
	No transplant shock	
	Automation	
	Easy packing and shipping	
Jiffy	No container to restrict roots	No media options
	Ready to go ex packaging	Negligible size options
	Preformed dibble hole option	
	High root ball integrity	
	Easy patching and sorting	
	No transplant shock	
	Automation	
	Easy packing and shipping	

***Pinus* x *attenuradiata* Hybrid Cuttings**

Field trials of *P. radiata* x *P. attenuata* hybrids, established by Scion and Proseed in the late 1990's, are showing them to be tolerant of cold and dry conditions and to have good resistance to snow.

The hybrid has rapidly become popular with companies for planting hard sites, particularly in higher altitude, snow prone areas. Popularity is now such that Proseed is unable to meet demand for seed. As practiced for similarly precious control pollinated *P. radiata* seed, Proseed has developed a technique to further bulk plants as rooted cuttings from stool plants.

In New Zealand, radiata pine cuttings are usually collected from open ground grown stools, once, in winter, and set directly into open ground beds under frost cloth.

Proseed has adapted another technique developed by Arbogen's Colac nursery in Australia. Potted stool plants are grown under cover and multiple collections of minicuttings are set year-round in cell trays, on bottom heat, under mist.

Whereas Colac set directly into the final growing container (almost 100% strike!). Proseed wanted to propagate in a smaller cell with a view to forwarding rooted plants to client nurseries for growing on. Small, rooted plants in a small, robust container would be better suited to machine/robotic handling and dispatch, so attention turned to propagation in plugs.

Cutting stools are grown in PBs in a crop cover tunnelhouse. Cuttings are collected to soak for at least 15 minutes in a bucket of IBA + NAA solution. When the bucket is full, the heap of cuttings is turned (so oldest are brought to the top) and cuttings are set into Jiffy Preforma propagation plugs. The plugs are supplied complete with a preformed dibble hole.

Cutting material is soft and requires frequent misting until cuttings harden and can maintain turgor again, usually 1 – 2 weeks. Misting is then dialed right back to irrigation once or twice a day. The Proseed misting system (a Multigrow controller by Autogrow) is driven by solar integral or timer or both.

Rooting is completed within 3 months of setting. A single collection from one mature, open ground grown stool might yield ~40 cuttings but, with this alternative system, a single stool might yield ~120 cuttings in a season.

While cuttings can be rooted year-round, bare root nurseries want plants for lining out October – November only. Storage of rooted plants through interim periods was an issue. Plants can be put out into a shadehouse and, without nutrients in the rooting media, hold reasonably well. Occasional foliar feeding helps keep condition without producing excess growth. However, the small plugs are vulnerable to rapid drying in hot weather.

Alternatively, plants can be held up to 7 months in cool storage without apparent undue effect. Storage in stackable "Lettuce" cell trays from Ellepot has proved both convenient (apart from a need for intermittent bottom watering) and effective (**Figs. 8** and **9**).

Proseed is currently producing up to 150,000 rooted cuttings a season. These are being supplied to a second party container nursery for growing on as soon as they are rooted, so storage has not yet been problematic.

Figure 8. Ellepot lettuce trays have proven useful for cool-storing rooted *Pinus x attenuradiata* cuttings. The trays are designed so that, when aligned the same way stacks nest into each other. But when trays in stacks are alternated end for end, stacks open up enough to accommodate small plants such as rooting pine cuttings in between.

Figure 9. A pallet of lettuce trays packed for cool storage. A shadecloth wrap (fastened with Velcro) proved better than film. The latter kept air inside humid and still, encouraging botrytis issues. Trays do require disassembly and bottom watering about once a week but have stored like this up to seven months with no adverse effect, either during or after storage.

Future Horizons – Tissue Culture and Plugs

As already stated, vegetative propagation of elite genotypes from cuttings affords fastest deployment and greatest genetic gain compared to propagation from seed. Tissue culture offers an opportunity to rapidly proliferate elite genotypes on a spectacular scale. For decades, plantation forestry has worked to realize this. Apart from difficulty, as with standard nursery techniques, of rooting older plant material, two persistent impediments to bringing such a process to scale have been cost of labour-intensive laboratory methods and transitioning soft plant material out of a sterile laboratory into a septic nursery environment. Recent technological advances seem finally to be bringing realization within reach.

Development of techniques to multiply seed embryos and advances in DNA analysis (not GM!) have led to genomics: a revolutionary new field of plant breeding. Instead of determining the breeding value of plus tree selections from the growth performance of their progeny, candidates can now be screened straight away for genetic markers associated with desirable growth traits.

Breeders are now moving to make forward selections even before seeds are fully mature, and to have a pathway that bypasses the adverse effect of physiological aging on propagation success.

For *Pinus radiata*, immature seed from elite pedigree crosses is dissected from green cones and callus is grown from somatic (undifferentiated) tissue. Some callus from selected genotypes (say 1,200 out of 10,000) is placed into cryostorage while other callus is matured to produce entire (shoot with root) plant embryos. The clonal embryos are “germinated” and grown on for further field testing to confirm and refine the genomic selections. Then, selected, still juvenile calluses are brought out of cryostorage and further proliferated for commercial establishment.

Rapid advances in automation and robotics look to be making bringing this technology to scale a real possibility. Examples include:

Bioreactors

In a bioreactor, tissue is placed on a plate in a tagine casserole like vessel that is periodically flooded with nutrient and hormone solutions to direct growth, either for callus or embryo development (**Fig. 10**).

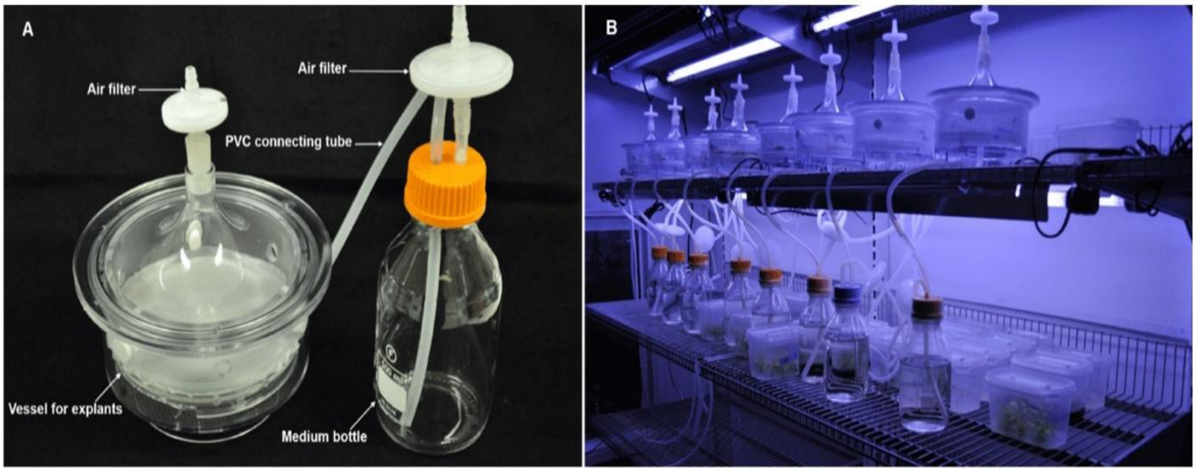

Figure 10. Use of liquid instead of a solid culture medium for the micropropagation of plants offers advantages such as better access to medium components and scalability through automation of the process. Scion Research has partnered with New Zealand Forest Industry to develop a protocol for large scale multiplication of selected *Pinus radiata* using temporary immersion bioreactors as pictured above. Compressed air drives nutrient and hormone solutions between the medium bottle and bioreactor, to bathe tissue cultures in the bioreactor. Bottles of different solutions can easily be changed as tissue growth develops.

Optical sorting of embryos

Embryos are placed into suspension and, through clever fluid dynamics, are sorted to single file past a camera for software to clean out and grade individual embryos through a high-tech drafting gate (**Figs. 11 and 12**).

One process is to drop each minute embryo onto a small filter paper that can be rotated to "right end up" and then gently folded into a mini taco to facilitate easy, automated handling for pricking out/germination.

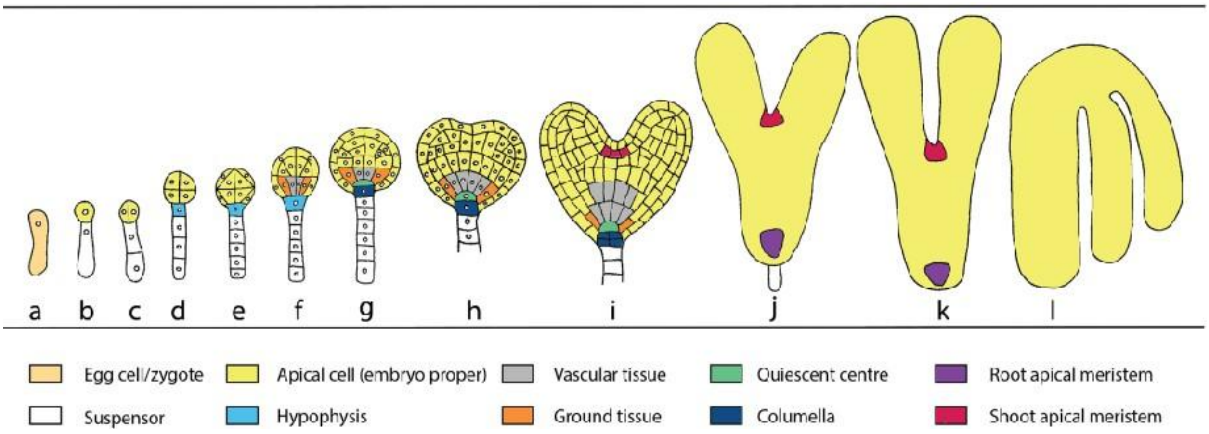

Figure 11. A schematic of seedling embryo development from fertilization of the egg cell through to a whole embryo seedling. Somatic embryogenesis begins with tissue harvested from the zygote at the very beginning of the development. Used with permission, M. Abrahamsson, Department of Plant Biology, Uppsala, Sweden.

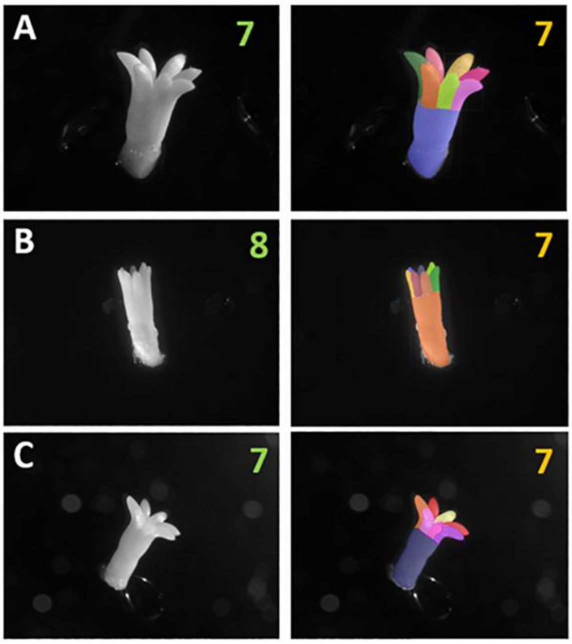

Figure 12. Computer images of embryos with a machine count of cotyledons shown down the left. Portions identified by the computer are colour coded on the right together with a manually corrected count.

Introducing the rightsoil flora

Protocols are being developed to prepare plants for a septic nursery environment by proper hardening and inoculation with healthy fungal flora before release from the laboratory.

Automation

Expectation is that most handling (pricking into plug trays, blanking, potting on) will be automated much as now with bedding plants provided to our box stores. Large forest nurseries already use automated systems for sowing and growing seedlings. Handling somatic embryos raised in plugs won't be too great a stretch for them.

For now, Proseed is watching this research work closely as, when successful, the implications for its business could be profound. Likely a move away from the large control pollination orchards and bulk collection it currently manages to perhaps smaller archives and fewer, carefully chosen controlled crosses from which developing seeds are taken for somatic embryogenesis. As an economic alternative to somatic embryogenesis, seed production from open pollinated seed orchards would likely continue. Meantime, it is prudent for Proseed to develop skills and experience in handling plants in plugs and trays since, like orchids, strawberries, and amenity plant varieties already, that is how the new tree breeding process will finish.

Growing Planting Stock for Native Forest Establishment

Currently there is repeated calling for New Zealand to reestablish native forest. That is canopy trees: not scrub but climax communities that used to be. MacFlora to go please.

Unfortunately, withstanding issues around planting and establishment, plant succession, biodiversity, ecosourcing, or what is a more effective carbon sink, for various reasons, from seed availability and/or dormancy through to specific growing conditions and sheer time to grow, nurserymen are finding many species, particularly some canopy species, difficult to grow. In general, smaller, pioneer species are easier, evidenced by their domination of highway and farm native planting to date.

Difficulty growing preferred, larger grade planting stock of native forest tree species translates to increased cost. However, buyers used to having exotic plantation species that grow to sturdy size in 1-2 seasons and retail around $1 each expect, and indeed need cost to be low. Trickier clonal stock like redwood and Leyland cypress can be had for around $5, yet some large grade native species retail for $20 or more, if they can be had at all.

If large scale planting of native canopy species is to fly, criteria include that cost of planting stock must be kept as low as possible. That means lifting plant percent (high germination/strike rates); shortest growing times; scaling up to spread overhead costs; and minimizing labour inputs through mechanization, automation and efficient handling systems. Plug systems offer remarkable opportunities to achieve all this (**Fig. 13**).

In support of Ngai Tahu interest in recloaking Papatūānuku (Mother Earth), Proseed has experimented with applying techniques described above (rooting mini-cuttings collected from a range of juvenile genotypes) to native forest tree species. Totara and kahikatea performed very well: near 100% and large cuttings quickly developing into large plants. Rimu, red and black beech didn't do nearly as well, though Auckland Regional Authority has reported good success with rimu in the past.

All food for thought re' bypassing tricky seed propagation issues.

In summary, propagation is pivotal in the future of forestry. To meet challenges sustainably and successfully that future will be highly technical and data intensive.

The future is exciting!

Figure 13. Rimu and totara cuttings 14 days after setting 8 June 2023 under mist. Larger cuttings in Transplant Systems TS45 cell trays and smaller cuttings in Jiffy Preforma plugs. The large group at left are on bottom heat while the cell trays at right have that turned off.

Figure 14. Totara tip cuttings from Transplant Systems TS45 in April 2024, 10 months after setting in June 2023 and potting up in January 2024, 8 months after setting.