Eucalyptus nitens breeding plan

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EXECUTIVE SUMMARY

Eucalyptus nitens breeding has a long tradition in commercial forestry in New Zealand. It is nowadays the most important *Eucalyptus* species in the country, and as a fast-growing species of good form, it would offer even greater opportunities to tree growers than currently is the case. The latest breeding plan update following the update in 2010 (Stovold et al. 2010) addressed the importance of developing this species towards solid wood production and consequently, proposed genetic research to gauge possibilities to improve these traits by breeding.

Recent genetics research showed good prospects for the genetic improvement of solid wood traits. Selections based on these studies have been made to the Specialty Wood Products (SWP) members' production seed-orchards. Additional selections will form a breeding archive and the basis for a new breeding population. These selections are proposed to be undertaken across three progeny trials in Southland (Keens Block, Fortification Road and Howdens Block) in this update to the breeding plan. Volume, wood quality, form and adaptability are all proposed as breeding objectives in this updated breeding plan. Selections at Keens Block will especially focus on improvement of wood quality whilst selections at Howdens Block (a subset of families) will be used to identify genotypes which have a better resistance to defoliating leaf beetles (*Paropsis charybdis*). Genetic diversity will be maintained by selecting at least 100 families, preferably 150 families based on estimated BLUP breeding values. Progeny from these selections will be planted for testing across two to three sites, if possible two in the South Island and one in the North Island.

The proof-of-concept genomic selection study undertaken in 2015 indicated that using markers gave considerably improved breeding value accuracies than pedigree-only based models. In addition, a business case developed to compare three different breeding scenarios, showed that forest growers will benefit of genomic selections through a predicted $7 million increase in the net present value compared with traditional breeding. It is therefore strongly recommended that the breeding of *E. nitens* take advantage of this opportunity through the application of genomic technologies in breeding in the future. However, further expansion of the measurements and genotyping to ensure more robust prediction models is required, including phenotyping more for solid wood properties and increasing the amount of genotyped individuals in the current population.

Essential operational actions in the breeding programme to target the breeding objectives and for implementation of this breeding strategy, as well as supporting genetic research are listed in this report.

INTRODUCTION

Eucalyptus nitens (Deane et Maiden) Maiden is the second most popular commercially planted eucalypt in its native country, Australia, and the most important commercially planted eucalypt in New Zealand. *Eucalyptus nitens* is described as a tall to very tall tree, usually reaching heights of 40 to 70 m and even as tall as 90 m with diameter at breast height 1 to 2 m and above (Boland et al. 1984). This forest tree species is recommended as the best species at sites of 500 to 700 metres in the Central North Island and is the eucalypt species most likely to be successful on most plantation sites in the South Island (Cannon and Shelbourne 1991). *Eucalyptus nitens* is presently distributed across the country, the major planting sites being located in the Southland and in the Central North Island (Meason et al. 2016).

Eucalyptus nitens was introduced to New Zealand in the 1920s and a breeding programme was initiated in the mid-1970s. The first cycle of a breeding programme targeted better growth, form and tolerance to environmental stresses. *Eucalyptus nitens* is a fast-growing species which with qualities for pulp-wood production and a great potential for solid wood production. In recent years there has been an increased interest around the world in breeding this species for solid wood products (e.g. Raymond 2002, Kube 2005, Kube and Raymond 2005, Biechele et al. 2009, Hamilton et al. 2009). *Eucalyptus nitens* has been mainly planted for pulp-production in New Zealand, however, developing this species for solid wood products, would ultimately provide scope for larger forest industry revenue via higher value timber products. The main challenge of *E. nitens,* as any other *Eucalyptus,* is in the efficient production of solid-wood products due to growth stresses that cause cracking and shrinkage of wood at drying, as well as timber movement on drying. Targeted breeding against these defects for better wood quality and improved conversation rates from log to lumber would permanently change wood property traits in the population. Significant breeding results will likely, however, require a long period of time (a rotation) to be observed in the harvest of the production population. In the meantime, and complementary to breeding, new wood processing methods may also offer efficient solutions for wood-drying problems by improving drying results for different solid wood products.

Eucalyptus nitens is recognised not only for its fast growth but also for good form and cold-hardiness. The first genetic studies for *E. nitens* in New Zealand focussed on testing the suitability of provenances across a range of sites (Franklin 1980, King and Wilcox 1988). Both these studies concluded that Central Victorian families had the best growth rates. King and Wilcox (1981) recommended that breeding should be based on the material originating from Central Victorian provenances which does, to a large extent comprise the current breeding population. As many as 96% of families in the 1990 open-pollinated population came from Central Victorian provenances which showed the greatest growth potential at the early breeding trials (Franklin 1980, King and Wilcox 1988).

The major limiting factor for the productivity of *E. nitens* is susceptibility to *Paropsis charybdis*, which is a defoliating *Eucalyptus* tortoise beetle (Wilcox 1980, King and Wilcox 1988). Currently, we do not know if any genetic factors play a role in susceptibility to *Paropsis* foliage damage. Franklin (1980) could see no reason to expect that seedlings of Victorian origin are any more susceptible to pests and diseases than those of New South Wales origin. However, a provenance trial planted on a warm site in Rotoehu forest in 1993 revealed better foliage health in provenances from New South Wales (Low 2000).

Breeding for improved resistance to browsing by *Paropsis* could offer an effective tool to enhance forest health and forest productivity. Identification of *Paropsis* resistant genotypes would require the development of field assessment methods that can measure variation in genetic analyses for observed phenotypes. Estimates of revenue loss due to pest damage in *E. nitens* are unknown and should be addressed through research in the future. Methods to assess resistance for the leaf-eating beetle as well as researching genetic background to resistance will be addressed through the Specialty Wood Products Research Programme and aim to target this challenge through breeding. A successful implementation of biocontrol using a natural enemy of *Paropsis*, called

Eadya paropsidis, would offer an environmentally friendly method to target to more resilient *Eucalyptus* forests in the short-term (Withers et al. 2015).

Another pathogen observed on warm sites is *Mycosphaerella*, a fungus that attacks *E. nitens* leaves in their juvenile state (up to five years of age). However, *Mycosphaerella* is not found on the majority of sites currently planted in *E. nitens*. The occurrence of the disease, however, may rise along the warming climate and therefore would be important to monitor whenever inspecting breeding trials.

The selection of the fourth generation is underway in the current breeding programme. Third generation progeny trials in Southland have been assessed for growth, form and wood density (Baltunis et al. 2013a, Baltunis et al. 2013b) and one of these was also assessed for solid wood properties (Stovold and Suontama 2015, Suontama et al. 2016). Selections for pulp and solid wood populations were made for South Wood Export's production seed-orchards in 2015 (Low et al. 2015). Research on new traits for solid wood production goals estimated good possibilities to improve trees by breeding based on considerable genetic variation between individuals. The objective of this paper is to update breeding objectives, selection criteria and strategy as well as next operational actions and contents of genetic research for *E. nitens*.

EUCALYPTUS NITENS BREEDING

Genetics research overview

Genetic variation in selection traits

One of the main interest in genetics research for this species is currently implementing the selection of solid wood traits into the selection schemes in order to improve wood processing and drying. New drying and processing methods offer additional, more rapid solutions for end-product users to mitigate effects of wood shrinkage and internal checking. Nevertheless, growth stress effects in wood are a combination of genetics and environmental factors such as silviculture practises (Kubler 1987) that can have a large effect on the development of growth stresses and related wood properties.

We estimated moderate heritabilities for traits important to solid wood production (growth strain, wood shrinkage and collapse, internal checking), showing good prospects for selection in this breeding population (Suontama et al. 2016). Since the wood properties are expensive to measure and development of non-destructive methods require a considerable amount resources, indirect selection by using surrogate traits such as volume or wood density can offer an option to expensive phenotyping. A correlated genetic response of wood properties would depend on the heritability of a trait and its genetic correlation with a surrogate trait. Due to a relatively small amount of data for quantitative genetic analysis, we were not able to conclude if indirect selection for expensive to measure wood property traits would be effective. However, estimated genetic correlations indicated that selection for some of the 'easily' measured traits should also result in an improvement in other traits of interest. Selection for wood density and stiffness should result in improvement of the quality of sapwood and heartwood (decrease in internal checking) (Suontama et al. 2016). Simultaneous selection against growth strain (split after sawing) and wood shrinkage would be possible due to favourable genetic correlations. It also implies that genetic gains would be achievable in both traits. The same study showed that ill-effects due to growth stresses and drying at different log lengths (at 3 and 6 metres) are likely to be determined by the same genetic pathways, based genetic correlations approaching unity. Consequently, selection could be carried out irrespective of log length.

New selection methods; Genomic selection (GS)

Eucalyptus nitens breeding strategy is based on an open-pollinated population that uses forwards selection with a rolling front strategy. A proof-of-concept study indicated that utilization of genomic selection (GS) in the estimation of breeding values would be useful for a new breeding strategy

(Klapste et al. 2016). The proof-of-concept study was carried out on the Keens Block progeny trial for growth, form and solid wood properties. The genotyping platform was a high-density single-nucleotide polymorphism (SNP) chip (EUChip) 60k developed for 12 different *Eucalyptus* species including *E. nitens* (Silva-Junior et al. 2015). The improved efficiency in breeding is established through two pathways in genomic selection. First, a possibility to decrease the time from selection to deployment by skipping a progeny trial assessment phase and selecting at the seedling stage will enhance genetic improvement (Resende et al. 2012). Second, genomic estimated breeding values would improve breeding value accuracies and the genetic gain available for selection, this is primarily due to the fact that genetic gain predictions based on open-pollinated tree populations lack the genetic information coming from a male parent.

Around 12,000 genomic markers were useful for the prediction of genomic breeding values in the *E. nitens* population after filtering which, based on statistical significance of a SNP marker in the study by Klapste et al. (2016). The accuracy of genomic predicted breeding values significantly surpassed the accuracies of BLUP breeding values in the proof-of-concept study for most of the traits (Klapste et al. 2016). Resende et al. (2012) reported that proportion of the accuracy of phenotypic BLUP selection recovered by GS in *Eucalyptus* varied from 0.75 to 1.20 depending on the trait and population in question.

Figure 1. Keens Block progeny trial in Southland where the tree material in the breeding population was evaluated for solid wood properties.

Economic analysis also supported the use of genomic selection, which was demonstrated in a business case for *E. nitens* based on parameters derived from this population (Corbett 2016). The business case analysis was built up for three breeding scenarios including traditional, genomics and genomics with clonal options for selection. Results indicated that using genomic selection, the net present value (NPV) results in $7 million increase for forest growers compared with traditional breeding. Further, the same study found that the NPV increases were even larger for end product processors, with an estimated gain of $34 million obtained for laminated veneer lumber (LVL) (Corbett 2016).

Genotype by environment interaction (G x E) in selection

The breeding strategy for *E. nitens* should be based on cost-effective methods due to a relatively small amount of planted area of this species in New Zealand, being currently 12,000 hectares. However, possible effects of genotype by environment interaction (G x E) on selections must be considered, ensuring new tree material will be tested in different environments if economically possible. Eucalypts are generally rather site-specific in their performance which can result in rank changes in breeding values estimation. This interaction between genotypes and environment impairs selection accuracy and consequently, results in decreased realized genetic gains. We estimated genetic correlations across the two progeny trials at Keens Block and at Fortification Road which indicated significant G x E for volume (0.29) (Table 1). Whilst other traits important to breeding objectives (DBH, wood density) did not express biologically important (genetic correlations were higher than 0.60) or statistically significant G x E. The two sites are located in a small distance apart in Southland, and these sites are likely to be characterised by similar site features. This may explain to some extent high/non-significant genetic correlations across two sites. On the other hand, the progeny trials shared only 29 families of the same origin in the parent generation that may affect the magnitude or significance of these genetic correlations as well.

The South Island has been traditionally considered as best suited region for planting *E. nitens* due to cold, wet conditions compensating problems with defoliating insects (King and Wilcox 1988), although the current knowledge declares that *E. nitens* plantations from Southland to the Central North Island can be heavily defoliated by *Paropsis* (Withers et al. 2015). There has been a recent interest in planting this species in the Central North Island (Withers et al. 2013) and some observations on young *E. nitens* have shown that it thrives at colder parts of the Kaingaroa Forest. It is therefore important to address, through genetics research, if there is a difference between genotypes in deployment at North and South Island sites. This should be done by testing the tree material of the similar backgrounds at least two or three more distant sites using the most common regions for the current commercial plantations of this species.

Table 1. Estimated genetic correlations (R g) with their standard errors (std. error) between two progeny trials in Southland.

Trait	R_g (std. error)
Height	0.40 (0.36)
DBH	0.38 (0.37)
Volume	0.29 (0.39)
Wood density	0.68 (0.28)
Malformation	1.00 (0.39)
Straightness	0.82 (0.18)

Breeding objectives and selection criteria

Two main production lines, one for pulp-wood to produce high-value paper products and another for solid-wood to produce sawn timber and appearance products, determine breeding objectives and selection criteria. Breeding goals are:

1) **Wood quality** which is defined by high wood density, low growth strain (growth stress), low shrinkage, a small number of or absence of internal checking and collapse (recovery after drying).
2) **Volume** defined as a function of diameter at breast height (1.3 m) and height.
3) **Form** defined as stem straightness and acceptability.
4) **Adaptability** defined as improved resistance to defoliation by insects.

The aggregate breeding value is a description of the overall breeding objective which comprises of goals 1) to 4). The overall aim is to deliver the tree material for the NZ forest industry that has a satisfactory wood quality for both high-quality solid wood and paper products, good growth and form and improved resistance to pests and insects (*Paropsis charybdis).*

Table 2. Breeding objectives and selection criteria.

Trait	Target	Impact
Wood density	Increase by 5 per cent Target population threshold 500 kg/ m^3	Increased yield of higher value pulp and solid wood
Wood shrinkage	Culling of genotypes above average rates of shrinkage	Better utilization of timber for specialty wood products and reduced processing costs
Internal checking	Culling of genotypes above average rates of checking	Better utilization of timber for specialty wood products
Volume yield	Increase by 5 per cent	More efficient production and lower costs to harvesting
Stem straightness	Maintain overall good form, cull all individuals below population avg. score 7	Better utilization of log at saw mill
Foliar health	Improved resistance to pests	Better growth rates and increased survival in different environments

The average values for growth and form and wood properties were described in Suontama et al. (2016) based on the assessments at Keens Block progeny trial at age six (growth, form, wood density) and age seven (wood density, shrinkage and internal checking). The average values for selection traits will be, however, re-estimated at the next phase of selections from that data which these next selections are based on.

Breeding strategy

Solid wood and pulp production seed-orchards established for South Wood Export
The forty-year-old breeding programme has undergone three rounds of selections. The current six breeding archives originate from the second generation progeny trials established during 1990-1992 (Figure 4). The breeding archive material was planted out to test progenies at three different sites in Southland between 2005 and 2011. In addition, a small subset of these seeds-sources was left and sown in Kaingaroa in 2011. All four trials partially share the same family material (Figure 4).

Two progeny trials, Keens Block and Fortification Road, were assessed at age six for growth and form and wood density (Baltunis et al. 2013a, Baltunis et al. 2013b). Age seven measurements for solid wood properties were undertaken at Keens Block (Stovold and Suontama 2015, Suontama et al. 2016). Based on these assessments, selections were carried out to establish new production seed-orchards at South Wood Export LTD. in 2015, first seed-orchard targeted for solid-wood production, and second seed-orchard targeted for high-yield pulp-wood production. At the first phase, the best genotypes based on BLUP (Best Linear Unbiased Prediction) estimated breeding values for DBH, wood density and growth strain (split after sawing) were selected for grafting. At the second phase, selections against wood shrinkage and internal checking were included in the seed-orchards. The clonal seed-orchards involve families which were assessed as the best material for the two production lines (Low et al. 2015).

Selection strategy
This updated breeding strategy will follow forest industry's demand to produce high-quality sawn timber and appearance products and high-quality paper (solid wood and pulp-chip). The breeding population will be managed as a single population with a 'rolling front strategy' using forward selection based on the estimation of individual tree breeding values (BLUP). A new breeding archive should be established by selecting 100 to 150 families to form the next generation's parents. The best families are recommended to be determined by ranking the families based on their estimated breeding values (progenies) and then selecting the best individuals based on their own estimated tree breeding values. Selecting an adequate number of families will ensure that genetic diversity is maintained and enabling genetic progress continues in the breeding programme.

The next selections should be conducted across the three latest progeny trials at Keens Block, Fortification Road and Howdens Block. Genotype by environment interaction was not significant across Keens Block and Fortification Road, except for volume. However, this should be explored, including Howdens Block, when the data is available. In case of no G x E across these sites, within-site selection can be undertaken. Otherwise, genetic correlations across sites should be accommodated into genetic model considering tree performances of similar genetic background as genetically different traits when exposed to different environments or site conditions.

Selections will be carried out by taking forward the best individuals within the best families and at minimum, covering 50% of the families from each origin (seed-orchard). Breeding objectives at the progeny trials will be emphasized as following:

1) Keens Block: **wood quality** (all traits), volume, form
2) Fortification Road: wood density, volume, form
3) Howdens Block: volume, form, **adaptability**

Forward-selected material from the breeding archive and production seed-orchards will be tested for breeding objectives at progeny trials when the seed is available. As stated earlier, 2 to 3 progeny trials will be planted to test the stability of tree performance in different environments. Trials should contain a set of the same families, at minimum 20 % of the same families but preferably much more, to have genetic connectivity between the sites accounting for genotype by environment interaction. Testing G x E is essential for new breeding objectives since this knowledge is not available currently in the breeding programme. However, no significant predicted G x E was found for *E. nitens* wood properties in Australia (Hamilton et al. 2009).

Developing cost-effective options to measure wood quality is necessary to address through research in the future. Use of NIR (Near Infrared Spectroscopy) to phenotype trees may offer a cost-effective and non-destructive option to maintain progressing in breeding for solid wood properties, i.e. shrinkage, collapse and internal checking. If non-destructive methods to measure wood quality do not prove to be reliable for breeding objectives, indirect selection for traits important to solid wood production should be sought after.

Figure 2. New production seed-orchards established at South Wood Export in 2015 targeting for improved solid wood properties.

Figure 3. *Eucalyptus nitens* progeny trial at Howdens Block in Southland was established in 2011. The tree material at this trial originates from Alexandra, Tinkers and Waiouru seed-orchards and is aimed at testing a sub-set of the families in the breeding population for resistance to the leaf-eating beetle *Paropsis.*

Genomic selection

Implementing genomic selection to predict breeding values of the tree material will be pursued in the breeding programme. Nevertheless, this requires collecting more material to 'validate' predictions for a wider range of phenotypes and genotypes to predict genomic breeding values at the seedling stage reliably. Klapste et al. (2016) emphasized the importance of capturing a large amount of genetic variation in training population to produce robust prediction models and proposed to keep the breeding population as a separate 'arboretum' to achieve genetic gains using genomics as efficiently as possible.

The implementation timeline will depend on the priorities and funding from the Specialty Wood Products Partnership and the Scion Strategic Investment Funding (the new name for Scion Core).

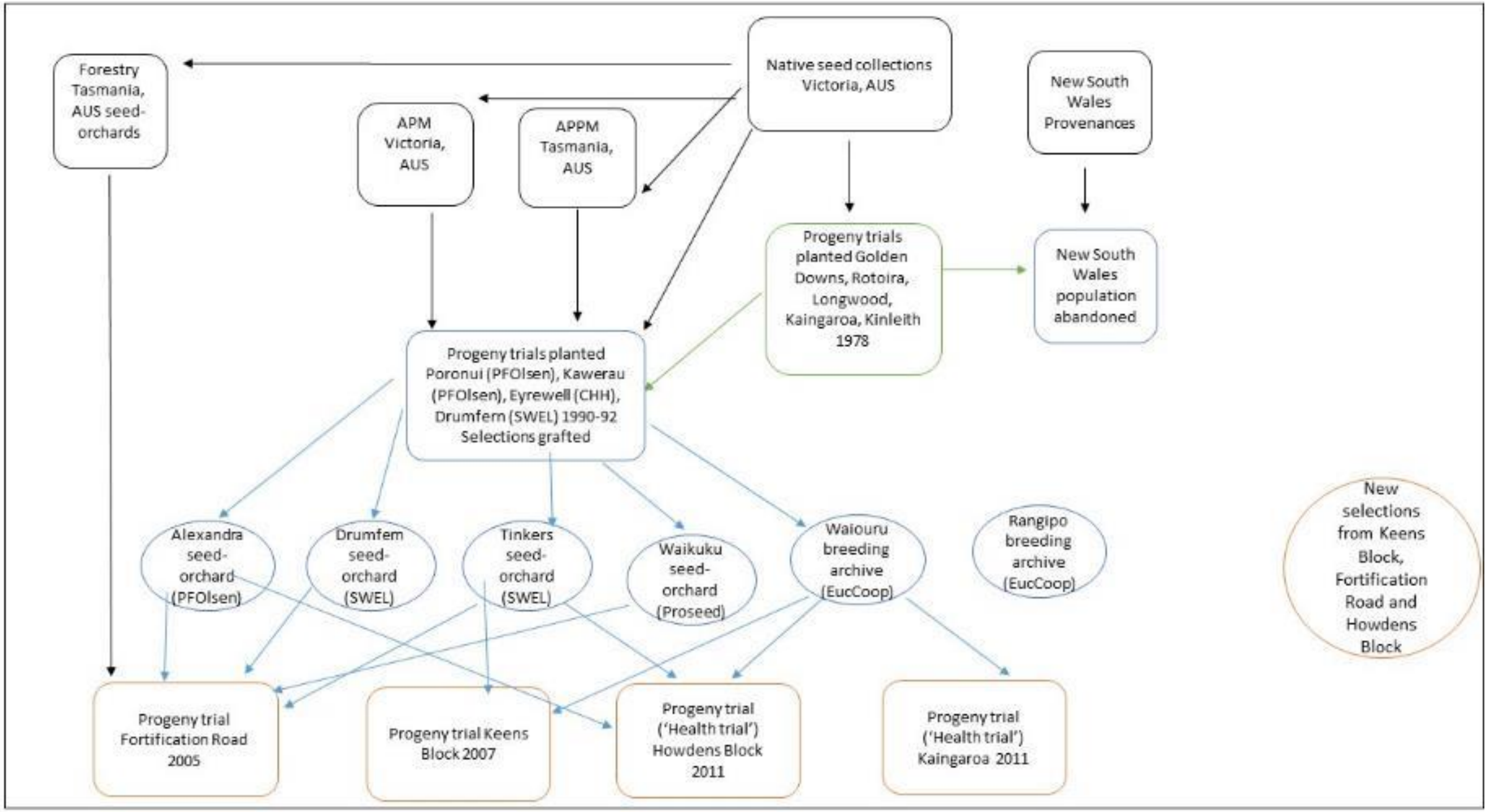

Figure 4. *Eucalyptus nitens* breeding programme. (After Stovold et al. 2010 and Baltunis et al. 2013).

Operational actions including genetic research 2016 onwards

All operational actions are subject to having sufficient funding. Work will be extended into additional financial years if this is the case.

2016-2017

Development of phenotypic assessment method for identifying *Paropsis* resistance
Assessing progeny trial at Kaingaroa (FR509) families originating from Waiouru breeding archive to develop a method to measure *Paropsis* damage.

2017-2018

Validation of phenotypic assessment method for *Paropsis* resistance and progeny trial assessment
The *Paropsis* assessment method developed at Kaingaroa will be validated at Howdens Block (containing offspring from Tinkers, Alexandra, and Waiouru seed orchards in Southland; pending the occurrence of insect damage in the trial). This trial was especially established for foliar health screening, so it has not been sprayed against insect/pests. These trees will be assessed also for growth and form to undergo a normal procedure at progeny trial assessment and to conduct selections for the next generation's parents.

2017-2018

Research on developing non-destructive methods to assess wood properties
Develop non-destructive methods to obtain a model that could be used to predict wood shrinkage and possibly internal checking by using NIR-scanner.

Aspirational goals for 2018-2019

New infusions of phenotypic and genotypic data for genomic selection
Collection of non-destructive measurements for solid wood properties at Fortification Road, subject to the availability of functional NIR correlation curves (we expect this will be completed in 2017-18). Material at Fortification Road will contribute additional seed-orchard material that is as yet untested for wood properties (Alexandra, Drumfern, Waikuku, Tinkers) in addition to the Keens Block genomics data already collected for which proof-of-concept genomic breeding values have been predicted (Tinkers, Waiouru). Additional genotyping to increase the total size of the genomic training population.

Aspirational goals for 2019-2022

Graft to establish breeding archive
Selections from progeny trials at Keen's Block, Fortification Road and Howdens Block will be grafted to establish a breeding archive.

The selections at the breeding archive and selections at the member's seed-orchards (South Wood Export LTD., Proseed) will be tested at 2 to 3 progeny trials, preferably one in the North Island and two in the South Island as soon as the seed is available.

Timeline for *E. nitens* breeding until 2022

2016

Selections made for production seed-orchards, updates to breeding plan completed.

2017

Development of assessment for pest tolerance. Production seed-orchards established.

2018

NIR methods to screen wood quality, validation of pest tolerance assessment method.

2019

Second set of genomic data and additional progeny trial data analysed.

2020

Screening of breeding population for natural tolerance to pests on-going.

2021

New selections for seed production initiated.

2022

Genotypes tolerant to pests identified, new progeny trials established, new selections grafted.

CONCLUSIONS

This breeding plan outlines updated breeding objectives for solid wood and pulp-wood production in *Eucalyptus nitens*. Breeding objectives for the two production purposes are wood quality, volume, form and adaptability. In addition to new selection traits for solid wood production, adaptability in terms of resistance to pests is considered as a critical trait to be updated in the breeding objectives as well. *Paropsis charybdis* is the major factor impacting productivity as well as interest in growing more this species in New Zealand. The breeding population will be managed as one population, but with selections able to be targeted at the two production purposes, pulp and solid-wood. The breeding strategy uses forwards selection in an open-pollinated breeding population, where the number of families maintained is at least 100 to 150 to ensure continuing genetic diversity and on-going genetic gains.

Genetic evaluation will be conducted using progeny testing and estimation of individual tree breeding values. Genomic selection options will be pursued in the breeding programme by additional phenotyping and genotyping to increase the robustness of prediction models. Progeny tests (trials) will be established at two to three sites which are regarded as commercially important forest growing areas for this species. Testing across multiple sites will elucidate the stability of genotypes across different environments, with specific attention paid to the plasticity of solid wood properties. Non-destructive, cost-effective methods to phenotype solid wood selection traits will be investigated. Investigation of the effectiveness of using indirect selection as an alternative to NIR methodology is also recommended.

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