Specialty Wood Products Research Partnership

SCION FORESTS ▪ PRODUCTS ▪ INNOVATION

Assessed defoliation of *Eucalyptus nitens* breeding population to quantify genetic basis of palatability to *Paropsis charybdis*

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FOREST GROWERS RESEARCH

TABLE OF CONTENTS

FOREST GROWERS RESEARCH

EXECUTIVE SUMMARY

Eucalyptus nitens breeding has a long history in commercial forestry in New Zealand. This is currently the most important *Eucalyptus* species in the country for pulpwood, and because it is fast-growing and has good form, it also offers opportunities for solid wood production. Essential operational actions in the breeding programme to target the breeding objectives and for implementation of a breeding strategy, as well as supporting genetic research, were listed in the SWP Technical report by Suontama et al. (2017) (SIDNEY 58661). In 2011, tree breeders decided they needed to understand the range of natural resistance, if any, present within the *E. nitens* breeding population to the most serious defoliating insect present in New Zealand, *Paropsis charybdis* (Coleoptera: Chrysomelidae). Seedlings resulting from open pollination of the 180 clones present in the breeding archive at Waiouru were planted out in Howden's block (FR507) on the Southwood Exports estate in Southland, as 30 replicates of single-tree plots (5400 trees). With these trees now eight years old, the appropriate time to assess the growth, and natural resistance to *Paropsis* had been reached.

As recommended in a previous literature review [Withers, Peters and Suontama (2017) SIDNEY 59185], in order to quantify the range in host palatability present, we needed to boost *P. charybdis* damage levels in *E. nitens* prior to assessment. In 2018, background populations of *P. charybdis* around Howden's block were still found to be low. The population was therefore supplemented by an adult beetle translocation from the central North Island. This was undertaken in November 2018 with approximately 2384 beetles, with an equal ratio of female to male adult beetles through sections one and two and a slight male bias in section three. Damage steadily built up over the growing season, as evidenced by three assessments conducted using the Crown Defoliation Index (CDI) method and ground observations. A final individual tree assessment was undertaken at the very end of the growing season, in late May 2019. Because of this and due to the height of some trees, the recommendation for assessing leaf number damaged and missing on the distal portion of the branch was not undertaken. Instead, defoliation assessments were limited to a qualitative visual rating of palatability scores (percentile scale of leaf retention from the previous growing season), completed consistently by the same person. At the same time, growth [i.e. diameter at breast height (DBH)] and stem form [i.e. stem malformation (MAL) and branching pattern (BR)] were also recorded.

There was considerable variation of palatability (defoliation from *P. charybdis*) scores, ranging from low to moderate (10-60%), resulting in low but statistically significant estimates of heritability (0.15) for resistance to *P. charybdis* feeding. In the future, an additional assessment will include the comparison between before- (December 2018) and after-treatment (May 2019) defoliation by quantifying crown width using remote sensing and analysis of individual tree crowns from UAV imagery, as well as ground-based assessment methodologies (T. Stovold SWP File Note 2019). Weather conditions were average for Southland over the growing season, but populations of *P. charybdis* may have been insufficient to exert sufficient defoliation pressure on the block, which may partly explain the low heritability estimates.

INTRODUCTION

Forest inventory data obtained from MPI (as of April 2016) showed that the total area of standing *Eucalyptus* spp. (Myrtaceae) in New Zealand was 23,182 ha out of a total exotic forest estate of 1,704,707 ha (NZ Forest Owners Association, 2017). However, MPI forest inventory data excludes small woodlot owners, who have less than 40 ha of forest. Smaller areas of eucalypts are known to occur commonly throughout New Zealand as shelterbelts, woodlots, in small groups, or individual trees. A conservative asset value of $671 million has been assigned to the value of this total *Eucalyptus* species forest estate (Radics et al. 2018, SIDNEY 61256). This could be increased in the future, if higher added-value end products than short-fibre pulp (e.g. wood flooring or ground-durable poles) are also produced from the existing *Eucalyptus* estate.

Eucalyptus nitens (Deane et Maiden) Maiden (Myrtaceae: Symphyomyrtus) is an economically important eucalypt species grown in New Zealand primarily for the production of wood chips for short fibre pulp, which is then either converted to high strength cardboard or exported for paper manufacturing (Haslett, 1988). There is a growing interest in its potential for solid wood (McKenzie, et al., 2003). Unfortunately, the pest *Paropsis charybdis* Stål (Eucalyptus tortoise beetle) (Coleoptera: Chrysomelidae) shows a strong feeding preference for *Eucalyptus nitens. P. charybdis* also finds most other species in the *Eucalyptus* sub-genus Symphyomyrtus highly palatable (e.g. *E. quadrangulata* H.Deane & Maiden white-topped box, and *E. globulus* Labill., Tasmanian Blue gum), but these species show slightly different susceptibility to the pest (White, 1973). It is also known that the proportion of susceptible species in different stands differs between regions. Regional MPI forest inventory data combined with Scion in-house species-site matching knowledge was used in 2018 to estimate the proportion of *Eucalyptus* species in each region that might be at risk from damage from *P. charybdis*. From this exercise, the weighted average across New Zealand of *Eucalyptus* plantations susceptible to *P. charybdis* was estimated at between 60-75% of the total. Therefore $400-$500 million worth of *Eucalyptus* stands are estimated to be at risk of being damaged by *P. charybdis* (Radics et al. 2018, SIDNEY 61256).

Apart from its popularity in New Zealand, *E. nitens* is the second most popular commercially planted eucalypt in its native country, Australia (Elek, 1997). It is described as a tall to very tall tree and is recommended as the best species for planting at sites above 300 metres above sea level in Tasmania, and sites above 500 to 700 metres a.s.l. in the Central North Island. *Eucalyptus nitens* is also the eucalypt species most likely to be successful on most plantation sites on the South Island of New Zealand (Wilcox, 1980). Trees that maintain a healthy crown have shown great potential as a provider of fast-grown hardwood timber in New Zealand. The early growth rate during the juvenile leaf stage can be impressive: up to 3 - 4 metres of height growth per year (Candy, 1997). *Eucalyptus nitens* is one of a number of eucalypts, along with *E. globulus,* that have distinctly different juvenile foliage for up to five years from seed germination (Brennan, et al., 2001; White, 1973). This juvenile foliage has a light blue colour, with short, waxy, almost round leaves that can cope with frosts of up to -10° C. The leaves of the adult foliage have a dark green colour and are long, narrow and sickle-shaped. The transition from juvenile to adult foliage can be easily observed. Unfortunately, *E. nitens* are susceptible to many leaf fungi and insect attacks (Hood, et al., 2002). Leaf fungi (e.g. *Mycosphaerella*) tend to devastate the juvenile leaf stage, while in New Zealand, most of the seriously damaging insects tend to prefer the adult foliage stage (Withers, et al., 2017). While one-off defoliation of the adult tree crown will rarely kill the trees, repeated attacks can dramatically decrease their growth and ultimately, economic value (Elek, 1997).

New knowledge has recently emerged from Tasmania, following long-term monitoring of *E. nitens* trial defoliation plots (Elek, et al., 2017). The most significant factors affecting the growth of the trees were the timing and frequency of defoliation. This research confirmed what was already suspected from anecdotal observations, i.e., the severity of a one-off defoliation event did not have significant effects over the long term. However trees that received either light or heavy defoliation late in the season for two consecutive years were at least 17% smaller in diameter, and mean annual increment (MAI) in diameter was reduced by at least 21% compared to untreated trees over

one rotation (Elek, et al., 2017). This now provides us with the evidence to justify the importance of managing pests to below populations levels ensure defoliation remains as light, or to develop a more durable tree resource that is resistant to attack.

Originally from Australia, *P. charybdis* was first recorded in New Zealand at Lyttleton Harbour near Christchurch in 1916 (Styles, 1970), then spread steadily until getting to the North Island by 1956 (Bain, et al., 1989). *Paropsis charybdis* thrived in the New Zealand *E. nitens* plantations due to the lack of any natural enemies to limit its population growth rates. *Paropsis charybdis* are found on *E. nitens* trees throughout the warmer months from October through to May and undergo at least two generations per annum. Both the beetle and its larvae feed voraciously on the flush of adult foliage so severe defoliation can occur during both during the early and late season, which was predicted by Elek and Baker to be the most serious times for the tree to experience a loss of leaf area. The use of biological control agents along with the aerial spraying of generalist insecticides have been the most common methods used by growers to control *P. charybdis* populations (Withers, et al., 2017; Withers, et al., 2013). Aerial spraying is, however, too costly for smaller plantations, and is not compatible with Forest Stewardship Council (FSC) certification, so it is unlikely to ever be considered as a long term management option for New Zealand plantations (Elek, et al., 2010; Rolando, et al., 2016).

The desirable properties of *E. nitens* along with the damaging effects that *P. charybdis* is known to be causing, motivated eucalypt growers and Scion, through the Forest Growers' 'Diverse Forests' programme, to further explore the natural resistance within the *E. nitens* families to *P. charybdis* attacks. In 2011, seeds of the improved breeding selection *E. nitens* families were obtained from the breeding archive at Waiouru and other seed orchards (Tinkers and Alexandra). These third-generation seedlings in the breeding programme were planted out in Howden's block (FR507) on the Southwood Export Ltd estate in Wairaki area, Southland, as 30 replicates of single tree plots (5400 trees), surrounded by a block of commercial *E. nitens*.

Intraspecific variation in the susceptibility of eucalypts to other pests has been explored elsewhere (Bennett, et al., 1992; Raymond, 1995; Richardson, et al., 1986). In the case of *P. charybdis*, the larvae cannot move from the tree on which they were oviposited by their mother as an egg batch. Therefore, the damage seen is a result of both adult feeding and larvae feeding on individual trees as a result of adult females making decisions on where to oviposit their egg batches, and for themselves, where they feed. We do not know what cues are used by most insects to make inter-tree choices, but in other *Paropsisterna* pests of *Eucalyptus* in Australia, a strong oviposition preference for certain *Eucalyptus* species or seedlots is expressed (Nahrung, et al., 2003). Antixenosis resistance is the host choice behaviour of an insect pest and usually is expressed as non-preference of the insect for a resistant plant compared with a more susceptible plant. The aim of this SWP program was, therefore, to test if any of the families in the *E. nitens* breeding populations induce antixenosis in *P. charybdis* for adult feeding and oviposition behaviour and/or tolerance to the resultant larval feeding. Should antixenosis be found to have a genetic basis, this will then be able to inform the *E. nitens* genomic selection programme in the future.

METHODS

Translocation and monitoring

Paropsis charybdis adults were collected from Poronui Station, east of Lake Taupo, New Zealand (-39.046905 S, 176.294889 E) during October-November 2018. Adults were obtained by beating infested *E. nitens* branches over a tarpaulin laid on the ground in the early morning. Adults would fall onto the tarpaulin where they could be caught by hand and put into aerated containers for returning to the laboratory (see photos in Appendix One). Captured adults were maintained on cut *E. nitens* branch stems with their cut bases sitting in water, in five, 60 L plastic containers, stored at 14˚C 16:8 L:D for a maximum of two weeks. Prior to release, individual adult *P. charybdis* were sexed based on tarsal morphology, colour and body size, then sorted into 4 pairs (4 females, 4 males) and placed in 50 mL ventilated plastic containers with one sprig of foliage and shipped in chill boxes to Invercargill overnight.

Release site and methodology
On the 31 October and the 1st November 2018 all beetles were released. The Howdens plantation has been planted in three similar sized sections divided into plots. Each plot had two containers therefore 16 beetles, released into it, the lid being taken off the container and the beetles shaken onto the base of the tree. Release trees in each plot were row two, on tree three, and tree seven. If the tree was dead, the beetles were released on the corresponding tree in row three.
A temperature recorder (iButtons, Maxim Technologies) was deployed in the middle of each of the three sections of the plantation.

Monitoring foliage damage
The Crown Damage Index (CDI) method was used to assess damage to adult foliage occurring in the Howden's experimental plantation (Stone, et al., 2003). Tree damage was assessed on three occasions during the growing season: in December 2018, and January and April 2019. For each assessment, six trees were selected ad-hoc from each of the three sections. Three branches were selected on each tree, one each at the lower, middle, and upper parts of the foliage. Assessors started at an edge for each section and worked towards the centre to account for edge effects. The CDI is made up of damage incidence (estimated percentage of leaves in the crown effected by each damage type) and damage severity (estimated percentage of how severely the leaves are affected by each damage type). Incidence and severity are usually recorded separately for juvenile and adult foliage. However, juvenile foliage was excluded here as only two trees were reported to have it present. Fungal damage was also excluded as it was also only reported on two trees. Instead, to calculate the CDI for each tree, the damage incidence and severity was multiplied for each of the lower, middle, and upper branches. Since no juvenile or fungal damage was included, the foliage type CDI is also the overall tree CDI here.

Foliage type CDI = (Lower branch insect incidence x Lower branch insect severity) + (Middle branch insect incidence x Middle branch insect severity) + (Upper branch insect incidence x Upper branch insect severity)

Tree CDI = Foliage type CDI

Genetic Analysis of Palatability and Growth
On the 20th of May 2019, 200 days after release of the translocated beetles, Scion staff assessed every individual tree on Howden's. The genetic evaluation was focused on the investigation of genetic parameters, in particular palatability of foliage to *P. charybdis* (PAL), which was recorded on a scale from 0 to 10. This scale reflects the proportion of current year's foliage in the crown lost to *P. charybdis* feeding, therefore zero represents an entirely untouched crown, and 10 a crown that has lost 100% of its foliage to chewing damage. Genetic correlations between PAL and other growth traits were also analysed. Growth traits included diameter at breast height (DBH), stem malformation (MAL) and branching pattern (BR) (both MAL and BR were measured on a scale from 1 to 9, where 1 is the poorest and 9 in the most preferable phenotype). All class variables (PAL, MAL and BR) were transformed to normal scores (Gianola, et al., 1981).

Analysis of variance components were performed by using linear mixed models implemented in the ASReml-R statistical package (Butler, et al., 2009) as follows:

$$\boldsymbol{y} = \boldsymbol{X\beta} + \boldsymbol{Zg} + \boldsymbol{Zr} + \boldsymbol{Zb} + \boldsymbol{e}$$

Where y is a vector of measurements, $\boldsymbol{\beta}$ is a vector of fixed effects such as intercept and position of the tree within a block (to correct for edge effect), $\boldsymbol{g}$ is a vector of random breeding values following $var(\boldsymbol{g}) \sim N(0, \boldsymbol{A}\sigma_g^2)$, where $\boldsymbol{A}$ is average numerator relationship matrix and σ_g^2 is additive genetic variance. The r term is a vector of random replication following $var(\boldsymbol{r}) \sim N(0, \boldsymbol{I}\sigma_r^2)$, where $\boldsymbol{I}$ is identity matrix and σ_r^2 is replication variance, and $\boldsymbol{b}$ is a vector of incomplete block nested within replicate effects, following $var(\boldsymbol{b}) \sim N(0, \boldsymbol{I}\sigma_b^2)$, where σ_b^2 is the incomplete block nested within replicate variance. The vector $\boldsymbol{e}$ contains random residual effects following $var(\boldsymbol{e}) \sim N(0, \boldsymbol{I}\sigma_e^2)$ where σ_e^2 is residual variance, $\boldsymbol{X}$ and $\boldsymbol{Z}$ are incidence matrices assigning effects from the vectors of fixed effects

$\boldsymbol{\beta}$, and random effects $\boldsymbol{g}$, $\boldsymbol{r}$ and $\boldsymbol{b}$ to measurements in vector $\boldsymbol{y}$. Narrow-sense heritability was estimated as follows:

$$h^2 = \frac{\hat{\sigma}_g^2}{\hat{\sigma}_g^2 + \hat{\sigma}_e^2}$$

Furthermore, the accuracy of breeding values estimates were estimated as follows:

$$r = \sqrt{1 - \frac{PEV}{\hat{\sigma}_g^2}}$$

where PEV is the prediction error variance (Mrode, 2014), estimated as the square of standard errors for breeding value estimates.

Genetic correlations were estimated by using a multivariate linear mixed model as implemented in the ASReml-R package (Butler, et al., 2009):

$$\boldsymbol{Y} = \boldsymbol{X\beta} + \boldsymbol{Zg} + \boldsymbol{Zr} + \boldsymbol{Zb} + \boldsymbol{e}$$

Where $\boldsymbol{Y}$ is a matrix of phenotypes. Since MAL showed no significant heritability in univariate analysis, it was removed from multivariate analysis to achieve model convergence. The matrix $\boldsymbol{\beta}$ contains intercepts and tree position effect for each trait, $\boldsymbol{g}$ is a matrix of additive genetic effects of each trait following $var(\boldsymbol{g}) \sim N(0, G1)$, where G1 is the variance-covariance structure for additive genetic effects $G1 = \begin{bmatrix} \sigma_{g_1}^2 & \cdots & \sigma_{g_1 g_n} \\ \vdots & \ddots & \vdots \\ \sigma_{g_n g_1} & \cdots & \sigma_{g_n}^2 \end{bmatrix} \otimes \boldsymbol{A}$, where $\sigma_{g_1}^2$ and $\sigma_{g_n}^2$ are the additive genetic variances for 1st and *n*th trait and $\sigma_{g_1 g_n}$ and $\sigma_{g_n g_1}$ are the additive genetic covariances between the 1st and *n*th trait, and $\otimes$ is a Kronecker product. The matrix $\boldsymbol{r}$ contains random replication effects for each trait, following $var(\boldsymbol{r}) \sim N(0, G2)$, where G2 is a variance-covariance structure for replication effects $G2 = \begin{bmatrix} \sigma_{r_1}^2 & \cdots & \sigma_{r_1 r_n} \\ \vdots & \ddots & \vdots \\ \sigma_{r_n r_1} & \cdots & \sigma_{r_n}^2 \end{bmatrix} \otimes \boldsymbol{I}$, where $\sigma_{r_1}^2$ and $\sigma_{r_n}^2$ are the replication variances for 1st and nth trait and $\sigma_{r_1 r_n}$, and $\sigma_{r_n r_1}$ are the replication covariances between the 1st and *n*th trait. Similarly, $\boldsymbol{b}$ is a matrix of incomplete block nested within replication effects for each trait following $var(\boldsymbol{b}) \sim N(0, G3)$, where G3 is a variance-covariance structure for incomplete block nested within replication effects $G3 = \begin{bmatrix} \sigma_{b_1}^2 & \cdots & \sigma_{b_1 b_n} \\ \vdots & \ddots & \vdots \\ \sigma_{b_n b_1} & \cdots & \sigma_{b_n}^2 \end{bmatrix} \otimes \boldsymbol{I}$, where $\sigma_{b_1}^2$ and $\sigma_{b_n}^2$ are the incomplete block nested within replication variances for the 1st and *n*th trait and $\sigma_{b_1 b_n}$ and $\sigma_{b_n b_1}$ are the incomplete block nested within replication covariances between the 1st and *n*th trait. Lastly, the matrix $\boldsymbol{e}$ contains residual effects for each trait following $var(\boldsymbol{e}) \sim N(0, R)$, where R is a variance-covariance structure for residual effects $R = \begin{bmatrix} \sigma_{e_1}^2 & \cdots & \sigma_{e_1 e_n} \\ \vdots & \ddots & \vdots \\ \sigma_{e_n e_1} & \cdots & \sigma_{e_n}^2 \end{bmatrix} \otimes \boldsymbol{I}$, where $\sigma_{e_1}^2$ and $\sigma_{e_n}^2$ are the residual variances for the 1st and *n*th trait and $\sigma_{e_1 e_n}$ and $\sigma_{e_n e_1}$ are the residual covariances between the 1st and *n*th trait. Genetic correlations between traits were estimated through Pearson's product moment coefficient:

$$r_G = \frac{\sigma_{g_1 g_2}}{\sqrt{\sigma_{g_1}^2 \sigma_{g_2}^2}}$$

where $\sigma_{g_1 g_2}$ is the additive genetic covariance between the 1st and 2nd trait, and $\sigma_{g_1}^2$ and $\sigma_{g_2}^2$ are the additive genetic variances for the 1st and 2nd trait, respectively.

RESULTS

Translocation and monitoring

The CDI scores within the plantation, following release of *P. charybdis*, were variable within each assessment, and across time. Over the growing season, the CDI scores steadily increased (higher damage incidence and severity) as *P. charybdis* populations grew, and adult beetles removed adult foliage (Figure 1). There were no obvious signs of edge effects, with damage evenly distributed across the edges and interior of the three sections of the trial. No fungal damage was recorded.

Because by the April 2019 assessment, the CDI scores for insect damage had reached over 100 (Figure 1), we assumed that sufficient damage had been inflicted by the *P. charybdis* to justify that the final analysis of palatability and growth should proceed.

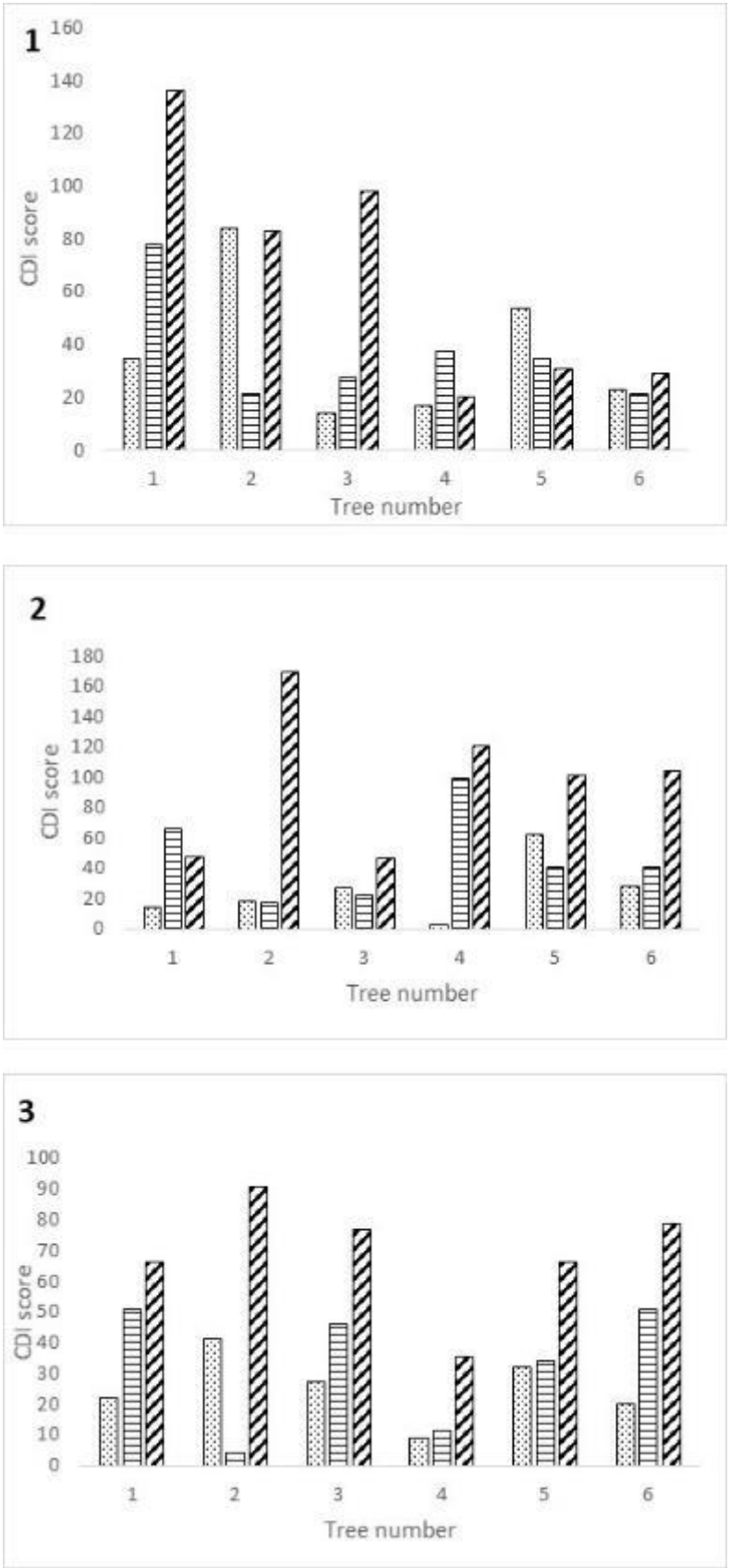

Figure 1. Crown Damage Index scores of the three sections of the FR507 Howden's *E. nitens*. trial.in Southland. Speckled: December 2018 assessment, horizontal lines: January 2019 assessment, diagonal lines: April 2019 assessment. The trend is for increasing CDI scores overtime in all three sections for the adult foliage. No fungal damage was recorded. Tree number refers to the six trees in each section selected ad-hoc for each assessment.

The temperature recordings over the season suggest that all three sections of the trial were experiencing similar temperature conditions (Figure 2). The temperature ranges suggest that the Howdens site experienced similar thermal conditions to those reported by NIWA for Southland (Fedaeff, 2019). NIWA reported that the 2018-19 summer was warmer than average (i.e. 0.5-1.0ºC warmer in Southland), as well as that the region experienced below-average rainfall (<50% of the summer average) (Fedaeff, 2019).

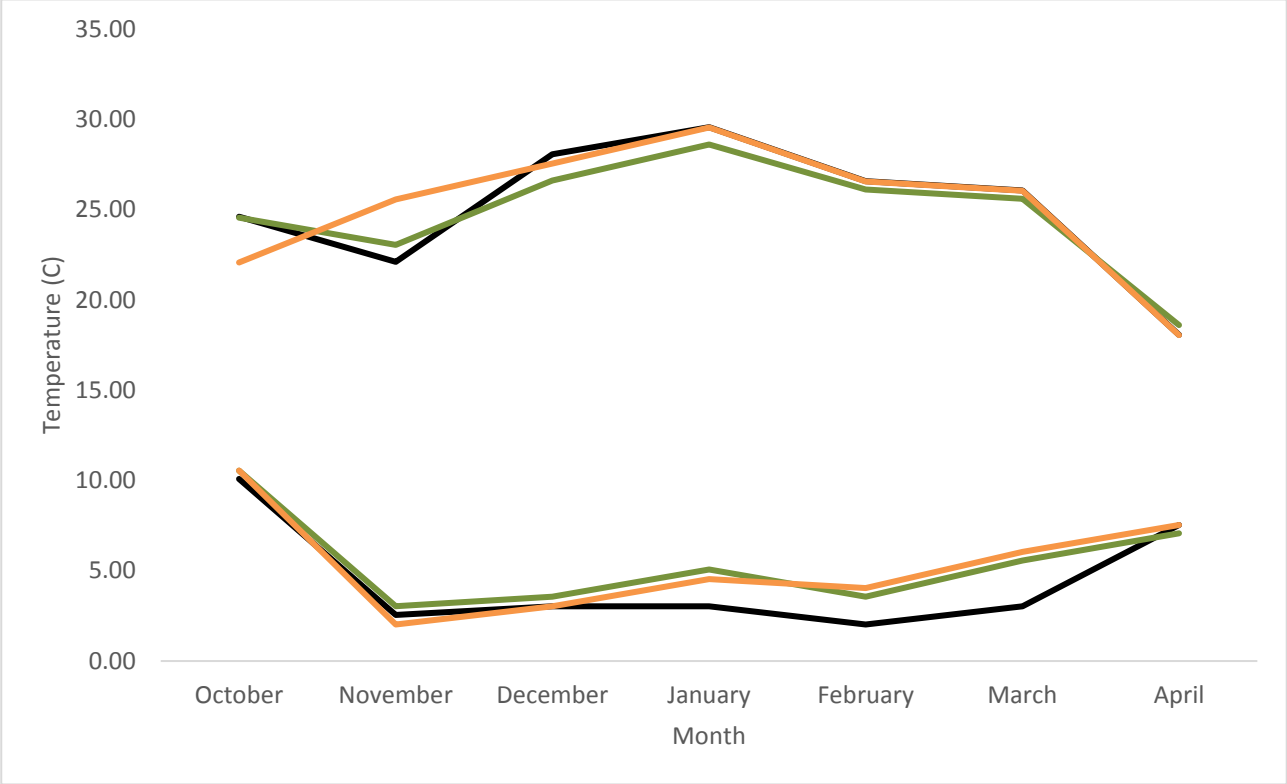

Figure 2. Monthly maximum and minimum temperatures from ibuttons suspended within the three blocks in Howdens, Southland. Black: block one, orange: block two, green: block three. Note that October and April data are only from measurements made on a single day.

Genetic analysis of palatability and growth

The assessment for palatability of the *E. nitens* to *P. charybdis* occurred approximately 200 days after the initial translocation. On average the mean PAL score was 3.09, (range low to moderate from 10% to 60%) and DBH was 210 (range from 9 to 380) indicating most trees had performed very well on this site (See Appendix two for photos).

The univariate mixed linear model analysis resulted in statistically significant additive genetic variances and narrow-sense heritabilities for most of the investigated traits, except for malformation. The narrow-sense heritability was 0.15 for PAL, 0.26 for DBH and 0.17 for BR, indicating the potential for genetic improvement through selection and targeted breeding. However, the level of heritability in *E. nitens* is commonly overestimated due to the high proportion of hidden relatedness and selfing. Therefore, the actual level of heritability may be substantially lower than our estimates. The accuracy of breeding values for heritable traits ranged from 0.46 (PAR) to 0.55 (DBH). These values are typical for open-pollinated field experiments (Table 1). There were statistically significant negative genetic correlations of -0.31 and -0.52 between PAL and the two heritable growth traits (DBH and BR), which indicated that smaller (or suppressed) trees were more attractive for *P. charybdis*. In addition, a strong positive genetic correlation was found between productivity (DBH) and branching pattern (BR) (Table 2). The distribution of parental breeding values within each seed orchard indicated that the families coming from the Tinkers seed orchard were the least productive and most sensitive to palatability (the highest breeding values). On the other hand, the material coming from NSW provenances (Waiouru_NSW) and the

Alexandra seed orchard showed the best productivity. However, only the NSW provenances showed lower palatability for *P. charybdis* feeding as well as high growth (Figure 3 and 4).

Table 1: Variance components, heritability h^2 (standard errors in parenthesis), accuracy of breeding value estimates (*r*) and model log-likelihood.

Parameter	PAL	DBH	MAL	BR
Additive genetic var.	0.041 (0.009)	350.0 (61.58)	0.00 (0.00)	0.088 (0.019)
Replication var.	0.150 (0.043)	103.7 (32.52)	0.42 (0.128)	0.045 (0.015)
Rep(Iblock) var.	0.054 (0.008)	51.7 (12.53)	0.021 (0.030)	0.029 (0.006)
Residual var.	0.236 (0.010)	1021.9 (56.33)	1.839 (0.082)	0.430 (0.019)
h^2	0.148 (0.033)	0.255 (0.043)	0.00 (0.00)	0.170 (0.035)
r	0.457	0.547	0	0.479
logL	384.2	-18323.9	-989.9	-908.5

Table 2: Phenotypic (above diagonal) and genetic (below diagonal) correlations (standard errors in parenthesis) between the investigated traits.

Trait	DBH	PAL	BR
DBH	1	-0.035	0.516
PAL	-0.306 (0.133)	1	0.04
BR	0.558 (0.093)	-0.52 (0.139)	1

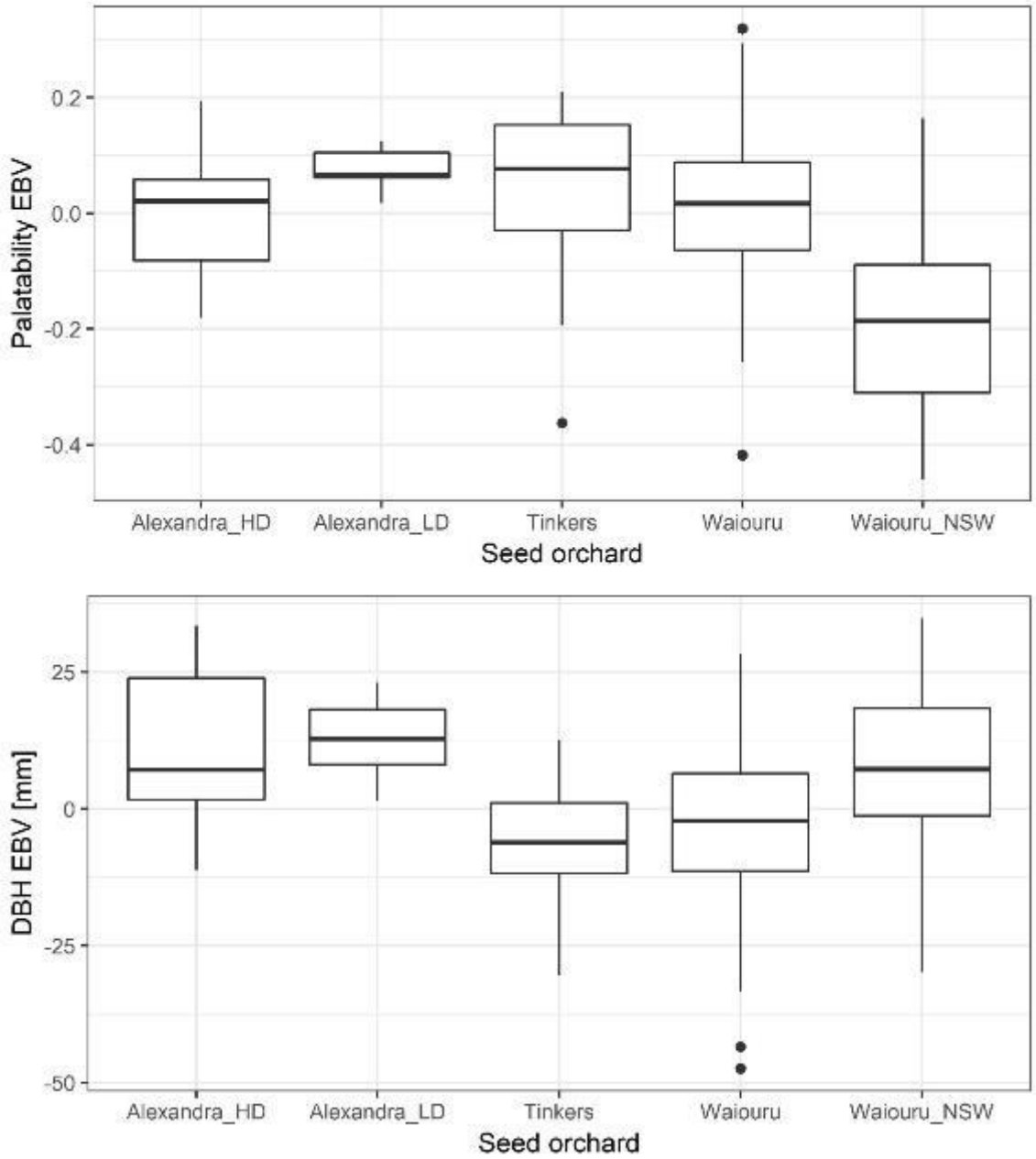

Figure 3: Distribution of estimated breeding values (EBV) for palatability (upper plot) and DBH (bottom plot) within each seed orchard (Alexandra_HD – selection for high wood density, Alexandra_LD – selection for low wood density, Waiouru_NSW – selection from material imported from New South Wales).

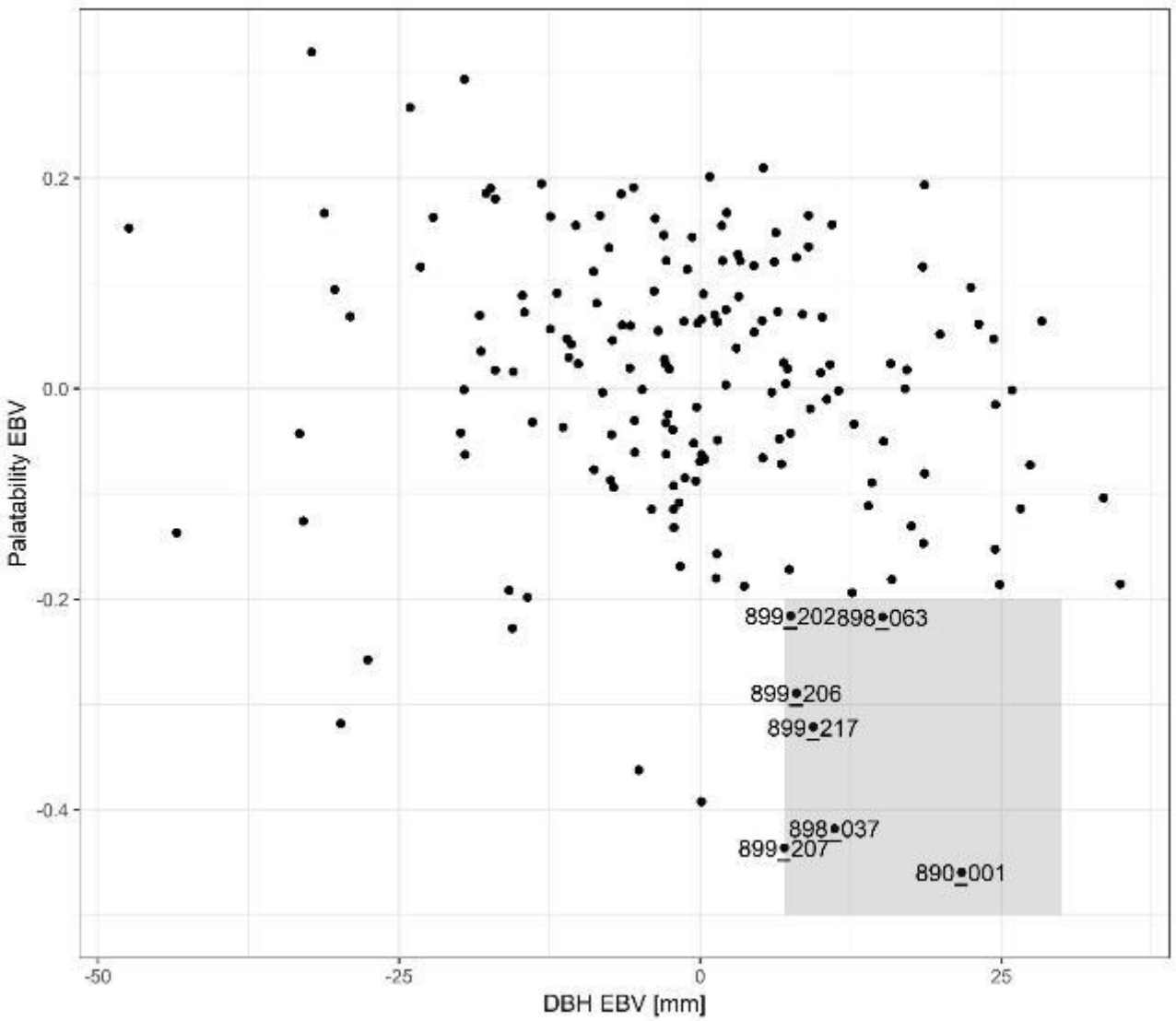

Figure 4: Correlation of estimated breeding values (EBV) estimated for DBH and palatability (mother trees having favourite combinations of breeding values are annotated). The shaded area highlights mother trees with lower palatability for *P. charybdis* feeding as well as higher growth (i.e. most desirable combination).

DISCUSSION

A significant level of genetic variation (heritability = 0.15) was found for palatability (levels of defoliation) in the *E. nitens* families growing in the trial (Table 1, Figure 3). The degree of observed defoliation, although only ranging from low to moderate, had a significant negative genetic correlation with DBH (-0.306), measured 200 days after the *P. charybdis* beetle translocation (Table 2). Thus, individuals with low productivity (suppressed trees, see Appendix two for an example) showed the highest degree of palatability. In this study, no measurements were taken prior to the introduction of the pests to the trial. However, we do not believe that the impact of feeding will have affected significantly the relative growth of the trees in the 6 months of the feeding having taken place. Instead, it appears likely that those trees were already suppressed for other reasons. Our analysis indicates that there is a direct link between growth rates during the first six years and susceptibility to defoliation. This may be because stressed trees put on less new growth in the current season, making feeding damage by *P. charybdis* more noticeable as it affects a higher proportion of the foliage. Whether this is related to the warmer than average summer temperatures and lower rainfall that Southland experienced, is not testable with the available data. Alternatively, beetles may be more attracted to suppressed trees for feeding or oviposition, or both. Whatever the cause of the correlation, the distribution of breeding values within each seed orchard clearly identified material from NSW as showing the most desirable combination of superior growth/highly productivity and low palatability (Figure 3).

Rapley et al (2004) reported that low heritability (range = 0.0-0.4) is commonly estimated for plant susceptibility to insect damage. Given the variation in the trial, open-pollinated eucalypt families must carry a range of phenotypic characteristic that lead to differences in antixenosis and, ultimately, palatability. Based on the observed defoliation patterns, we suspect that the adult *P. charybdis* could be differentially attracted to some of the *E. nitens* families for feeding and also for oviposition, with a clear preference for families with many suppressed individuals. The *P. charybdis* adult beetles feed upon the flush adult foliage (i.e. the new rapidly expanding red-coloured leaves which are rich in secondary chemicals). This type of foliage is also vital for the adult females, which initially emerge from over-wintering teneral (softened), without matured ovaries, to feed on to become fecund (Bain, et al., 1989). However, it is not these leaves that the adult lays her egg rafts on, as this would increase the risk of the eggs being eaten by conspecifics. Instead, the adult female, when her ovaries are mature and she has been mated, moves down the tree to unpalatable old, hard foliage and lays her egg rafts there. The hatching neonate larvae then undertake positive phototaxis and move to the tips of the branches to again locate the flush foliage that their minute mouthparts are only able to feed on. In this way, it is the combined feeding pressure from both adult females undertaking maturation feeding, and their newly hatched larvae, that creates the defoliation we have assessed in this trial (Styles, 1970).

It has been suggested that leaf colour is a visual attractant to paropsine beetles, the red colour typical of the flush expanding leaves being a predictor of attraction in *Paropsisterna bimaculata* (Olivier) (Raymond 1995). In many cases, the mechanism for the variable attraction is uncertain. For instance, feeding trials revealed significant differences in the attractiveness of geographic races of *E. globulus* for oviposition by the autumn gum moth *Mnesampela privata* Guene´e (Lepidotera: Geometridae), but there were no significant differences between families within races (Rapley, et al., 2004). Steinbauer et al. (2004) have hypothesised that foliar attraction to autumn gum moth may be related to various concentrations of foliar monoterpenes. Similarly, Jones et al. (2002) reported that oviposition deterrences were associated with levels of several aliphatic phenylethyl and benzyl alkanoates found in *E. globulus* foliar waxes.

Even in the absence of knowledge of what mechanisms might be guiding a pest like *P. charybdis* to differentiate between families of *E. nitens* for feeding and/or oviposition, it is still possible to improve screening and deployment strategies in New Zealand (i.e. because there are heritable differences in palatability between families). Breeding for resistance could potentially be accelerated, if the mechanisms underlying feeding and oviposition preferences were understood better. However, we can still use the variability within the breeding population to prioritise fast-growing families that also have lower palatability to *P. charybdis*.

CONCLUSION

Our analyses resulted in a low, but statistically significant estimate of heritability (0.15) for the palatability of *E. nitens* to *P. charybdis*. In addition, there was a negative genetic correlation between palatability and productivity (DBH). Thus, suppressed individual trees had the highest level of palatability or feeding damage. However, the strength of the genetic signal may be driven by the level of phenotypic variation. Therefore, repeating this study during a year with higher population density of *P. charybdis* could be beneficial to our understanding of the genetic determination of palatability.

The analysis of parental breeding values within each seed source (seed orchard) suggested that material from New South Wales provenances appeared to be well adapted to South Island environmental conditions (i.e. had higher growth) and also had lower palatability to *P. charybdis*. Individuals with both good growth and apparent resistant to browsing could be selected in this trial. Comparing these with genotypes in the orchard with a view to rogueing susceptible genotypes and/or augmenting the orchard with these resistant genotypes is recommended.

A recommendation would be to monitor the trial over the summer of 19/20 and if more chewing occurs look to undertake a second assessment to confirm the most suitable trees. Finally, more precise phenotyping platforms based on remote sensing would help to improve phenotypic differentiation between families, leading to more precise selection decisions in a breeding program for resistance to *P. charybdis*.

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Appendix One: Translocation photos showing methods used to collect *Paropsis charybdis* in October 2018 and prepare for shipping to Southland

A typical collection tree at Poronui Station (left) for beating and shaking the foliage, and adult beetles able to be collected on a tarpaulin spread beneath the tree (right).

Adult beetles stockpiled in large containers with *E. nitens* foliage in the laboratory (left) and separated into n=4 pairs in small containers for shipping to Southland, 30 Oct 2019 (right).

Appendix Two: Photographs of trees in Howden's, May 2019

Examples of suppressed (left) and healthy (right) *E. nitens* during the assessment at Howden's.

Examples of *E. nitens* trees during the assessment at Howden's, some notching of leaves from *P. charybdis* feeding is evident.

Appendix Three: Method for monitoring the progress of defoliation in Howden's through the season following translocation

Crown Damage Index

The Crown Damage Index (CDI) is a visual assessment of the entire tree undertaken from the ground. The method was developed to provide a standardised, repeatable and statistically valid method to record pest and disease damage on **young** *Eucalyptus* trees (refer to the full publication in Stone et al. 2003). The CDI was designed to provide a method that was relatively quick and simple to apply, where the measure of damage could be easily summarised, and be reasonably reliable between observers.

There are seven steps to assessing the CDI of a *Eucalyptus* tree (Stone et al. 2003). These are:

Step 1: A walk through the whole plantation before the commencing of the sampling to familiarise the assessor with the range of conditions that exist at the site.
Step 2: Andrew has chosen a systematic walk following a particular direction and assessing six trees. The assessor should view the tree they are specifically assessing from more than one side to identify the type of damage present on the tree.
Step 3: Calculating the damage incidence of the tree: The damage incidence is the estimated percentage of leaves in the crown effected by the type of damage that is being observed. The damage incidence can be calculated specifically for different parts of the crown to improve accuracy. More detail, including diagrams and examples are presented in the CDI manual (Stone et al. 2003).
Step 4: Calculating the damage severity of each tree. The damage severity is the estimated percentage of how severely the leaves are affected by the type of damage that is being observed. Like the damage incidence, the damage severity can be calculated differently for different parts of the crown to improve accuracy.
Step 5: Enter the incidence and severity score for each tree into the excel sheet provided, these two scores are multiplied together and divided by 100, to give a continuous variable between 0 and 100.
Step 6: The CDI of a tree is determined by adding each incidence x severity product together.
Step 7: It is also essential to take note of the date of the measuring of the CDI and what the damaging agents observed actually were, in the case of Howden's it is imperative the observers can tell the difference between fungal leaf spots and leaf blisters or chewing caused by insect agents. We will ignore the presence of psyllids within tips if they are not doing any damage and ignore *Eriococcus* on twigs if they are also not doing any damage.

Table 1. Examples of insect damage you may see in Southland.

Table 2. You need to learn to separate Insect spots and fungal spots – in this case both are estimated at 5% severity and 5% incidence.

Table 3. Typical fungal leaf spots with individual leaf severity of fungal spots.

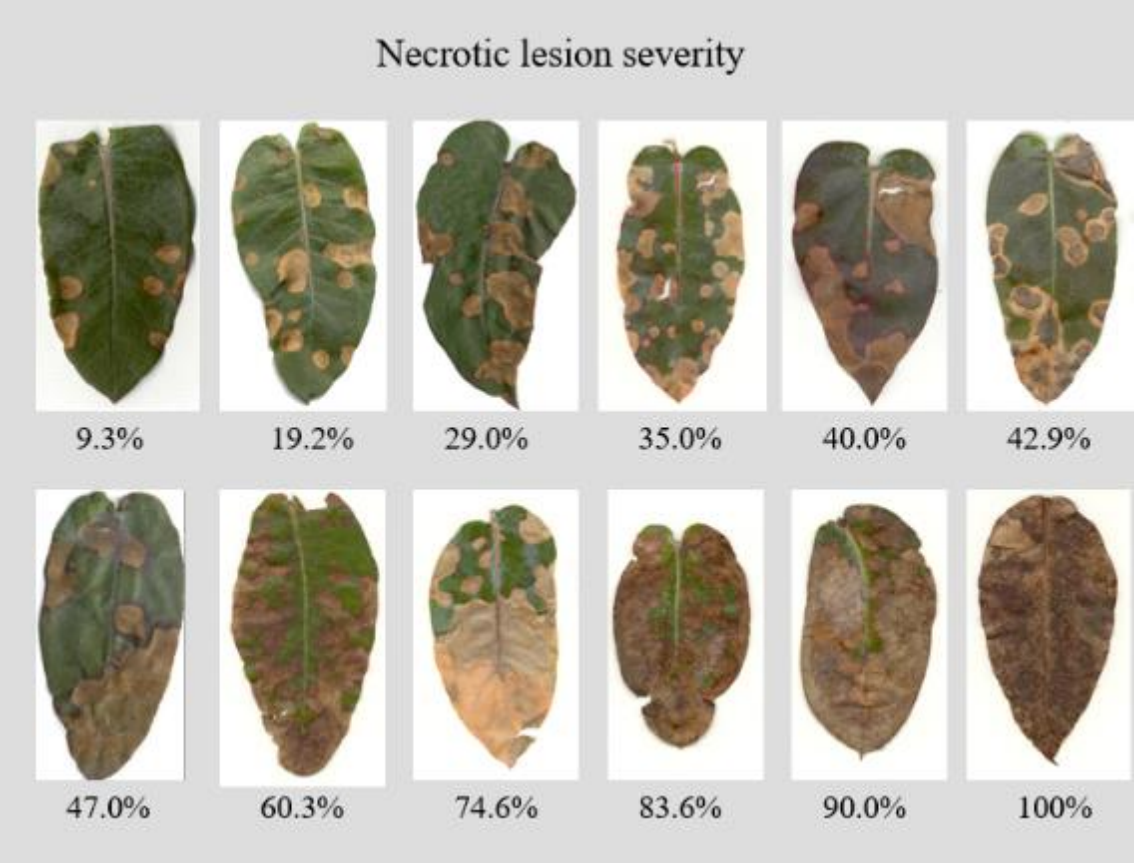

Table 4. Examples of how to come up with a mean fungal spot severity: both of these examples would be given an incidence of 90% (9 out of 10 leaves affected in some way) and a fungal severity of mean 40%.

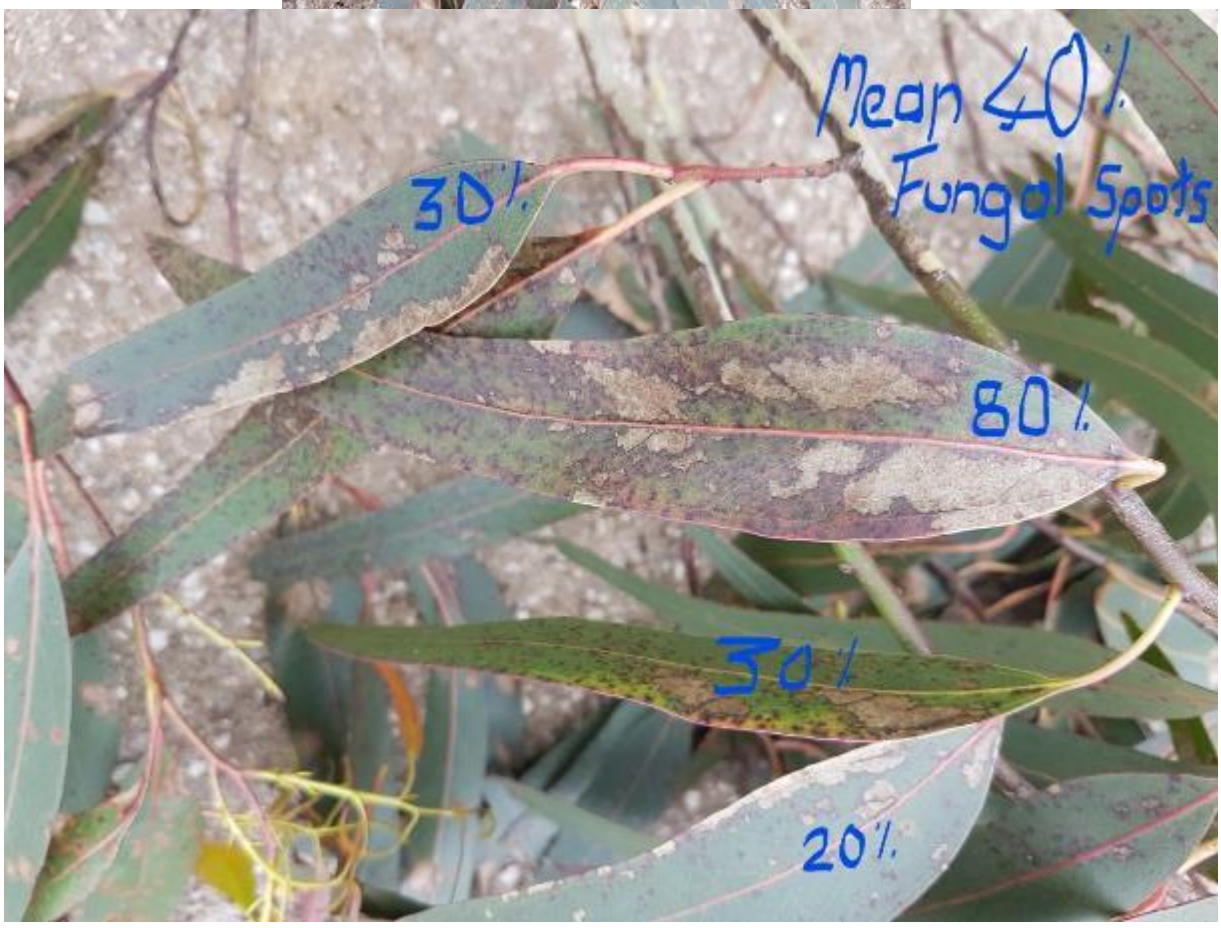

Table 5. Example of 8% fungal severity, with spots seen on both older and newer foliage.