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Review of methods and recommendation for assessing defoliation *Eucalyptus nitens* from *Paropsis Charybdis* for breeding purposes

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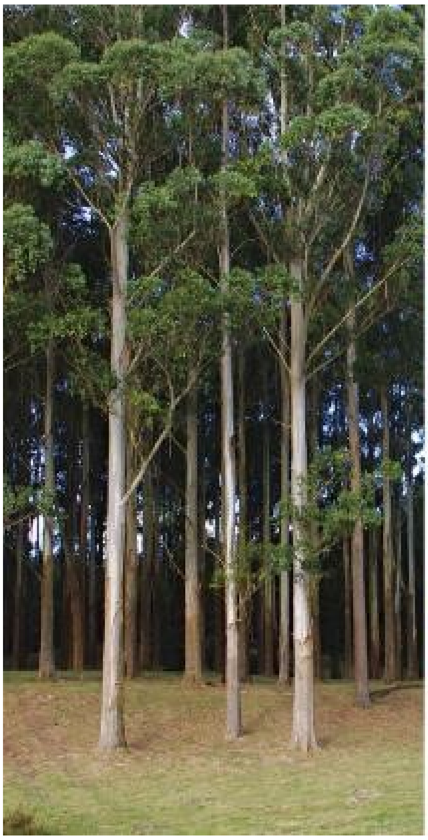

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FOREST GROWERS RESEARCH

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EXECUTIVE SUMMARY

Eucalyptus nitens has a long tradition in commercial forestry in New Zealand. It is currently the most important *Eucalyptus* species in the country. As a fast-growing species of good form it could offer even greater opportunities to tree growers for solid wood production, in addition to its current use for pulpwood. The Eucalyptus tortoise beetle, *Paropsis charybdis*, remains the most serious pest of *E. nitens* in New Zealand. In 2011, tree breeders agreed that the natural resistance of *E. nitens* to *P. charybdis* attack should be further explored. As a result all 180 families (excluding the Tasmanian material) from the New Zealand breeding population was collected and sown. The resultant seedlings were planted out in Howdens block (FR507) on the Southern Wood Export estate in Southland as 30 replicates of single tree plots (5400 trees). These trees are now approaching six years of age and breeders want guidance on a method to assess the growth, form, wood density, and resistance to *Paropsis*.

A literature review and an assessment of a subset of trees in a small plot (FR509) in Kaingaroa Forest were used to evaluate different methods of scoring resistance to *P. charybdis*. Methods tested were single branch observations, crown damage index, *Paropsis charybdis* chewing score and crown density rating to assess *P. charybdis* damage in the breeding population in Howdens block.

Based on field trials and literature review, we recommend the following:

1. Supplement natural populations of *P. charybdis* at Howdens block by releasing approximately 2000 live adults into the plantation, in spring 2017. Higher population levels are required to assess defoliation score resistance of individual trees (to be greater than an average of none or trace). After assessment, an aerial spray may be required in late summer to prevent long-term pest impacts.
2. In late summer, climb each tree 1m and using extended electric pole pruners obtain one random sample of an adult foliage-bearing branch to quantify the proportion of adult leaves damaged by chewing and/or missing from the terminal 100cm. Calculate a branch chewing score. Photograph any live insects still present on the branch and record photo number for later identification.
3. Undertake a qualitative late summer visual rating of upper crown density on a 1-5 scale (*Paropsis* chewing score). There is potential to obtain an aerial drone photograph of each tree to compare rating to a visual image.

Assessment of the *E. nitens* breeding population at Howdens block using these recommended procedures should be undertaken before the trees grow any taller as their crowns become inaccessible. Furthermore, earlier testing provides faster decisions for the next generation of breeding trials.

INTRODUCTION

Eucalyptus nitens (Deane & Maiden) Maiden is the second most popular commercially planted eucalypt in its native country, Australia, and currently is the most important for New Zealand. *Eucalyptus nitens* is described as a tall to very tall tree, and is recommended as the best species for planting at sites of 500 to 700 metres a.s.l. in the Central North Island, and is the eucalypt species most likely to be successful on most plantation sites in the South Island (Suontama et al. 2017).

Eucalyptus nitens trees that maintain a healthy crown have shown great potential as a provider of fast-grown hardwood timber in New Zealand. Early growth rate can be impressive at 3-4 metres of height growth per year (Suontama et al. 2017). *Eucalyptus nitens* is one of a few species, along with *E. globulus,* that has distinctly different juvenile foliage for up to five years from seed germination (Brennan et al. 2001). This juvenile foliage is 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 are a dark green colour, long, narrow and sickle shaped. Also adult foliage nodes are alternate rather than opposite. The transition from juvenile to adult foliage can be easily observed. *Eucalyptus nitens* has adapted to an alpine climate with winter frosts and snow, with typically hot, dry summers. The natural range exists at altitudes of 600 to 1600 metres asl from northern New South Wales at latitude 30°S to Victoria at latitude 38°S. The climate in most New Zealand forests is rather different, being influenced by proximity to the sea to be more humid in summer and warmer in winter with infrequent snow. Unfortunately *E. nitens* trees are susceptible to many leaf fungi and insect attacks especially when grown on warmer sites (Hood et al. 2002). While defoliation of the crown will rarely kill the trees, these attacks can dramatically decrease their growth and ultimately the economic value.

Economic losses that can be blamed on the effects of just one pest or pathogen have been difficult to quantify in New Zealand (Bain & Kay 1989) but new knowledge has recently emerged from Tasmania following long-term monitoring of trial defoliation plots. The most significant factors affecting the growth of the trees were timing and frequency of (artificial) defoliation. The severity of a one-off defoliation event did not have significant effects over the long term. Trees that received either light or heavy defoliation late in the season for two consecutive years were at least 17% smaller in diameter with the mean annual increment (MAI) in stem diameter being reduced by at least 21% compared to untreated trees over one rotation (Elek & Baker 2017).

The most significant pest insect currently damaging *E. nitens* in New Zealand, is the Eucalyptus tortoise beetle, *Paropsis charybdis* Stål (Chrysomelidae). Unlike the major defoliating beetle in Tasmania, *Paropsisterna bimaculata*, that feeds on juvenile foliage, the pest *P. charybdis* in New Zealand feeds on the flush of the adult foliage, and finds juvenile foliage unpalatable. Since 2017 two more similar Australian species of paropsines, *Paropsisterna beata* (Newman) (Yamoah et al. 2016), and *Paropsisterna variicollis* (Chapius) have also been confirmed as established in New Zealand, in Wellington, and Hawke's Bay bioregions of the North Island, respectively. Both species are also known to feed on *E. nitens*. Of these two recent invaders, *Pst. variicollis* is predicted to have the greatest impact, and in the future may rival *P. charybdis* in pest status (Lin et al. 2017).

Originally from Australia, *P. charybdis* was first recorded in New Zealand at Lyttelton Harbour in Christchurch in 1916 (Styles 1970) making its way to the North Island by 1956 (Bain & Kay 1989). *Paropsis charybdis* thrived in the New Zealand *E. nitens* plantations due to the lack of any natural enemies that limit population growth rates in its native range. *Paropsis charybdis* is found on *Eucalyptus* trees during the summer, and undergo two generations per annum. Both the adult beetle and its larvae feed voraciously on the flush of adult foliage. Numbers build over subsequent years and plantations suffer heavy defoliation a few years after the trees transition to adult foliage.

The use of biological control agents along with the aerial spraying of generalist insecticides have been the most common method used by growers to control *P. charybdis* populations (Withers et al. 2013). But aerial spraying is costly, and incompatible with Forest Stewardship Council (FSC) certification, so is not a long term management option for all plantations in New Zealand (Elek & Wardlaw 2010; Rolando et al. 2016).

The desirable properties of *E. nitens* and the damaging effects that *P. charybdis* is having, prompted the *Eucalyptus* Breeding Co-operative to explore natural resistance of the *E. nitens* families to *P. charybdis* attack. In 2011, seeds of the improved breeding selection *E. nitens* families were obtained from the breeding archive at Waiouru and other seed orchards. To test resistance in the breeding population, 30 replicates of single tree plots (5400 trees) from the third generation seedlings were planted in Howdens block (FR507), South Wood Export estate, Southland. A sub sample of seedlings from 32 families were planted at the same time, as 2 replicates each of 9-tree row plots in Kaingaroa Forest compartment 638 (FR509) (Timberlands Ltd) surrounded by a block of commercial *E. nitens*.

The aim of this report is to review the literature on assessment methods and provide recommendations on the best method to assess *P. charybdis* damage levels. The aim is to include the method(s) recommended from this report in future breeding programme practices for genetic analysis. This will allow susceptibility to *P. charybdis* to be included in the future genomic selection programme for *E. nitens* in New Zealand.

METHODS

REVIEWED SAMPLING METHODS

Four methods from the *Eucalyptus* literature that were thought to be the most suitable candidates for assessing *P. charybdis* damage on *E. nitens* in New Zealand were examined. These methods (summarised below) included single branch observations, crown damage index, *P. charybdis* chewing score and crown density rating. Not all methods were trialled experimentally in the assessment of FR509 in 2016/17 as *P. charybdis* population levels were low and browsing damage was minimal.

1. Single Branch Observations
It is possible to quantify insect (or fungal) incidence in a *Eucalyptus* stand by assessing a proportion of trees from each seedlot and assessing a fixed number of branches per tree. Percentage leaf retention and percent infection incidence are recorded (Carnegie et al. 1994). For example, Hood et al. (2002) assessed four branches (one on each compass quadrant) per tree, and evaluated 32 or 48 trees per seedlot for fungal infection. For many fungal leaf spots this is achieved by inspecting juvenile leaves, however assessments of insect pest abundance must examine adult leaves. This is only practical in NZ for *E. nitens* trees < 5 years old or < 9 metres in height. Field scouts in Australia assess the terminal 50cm of a branch bearing juvenile leaves and count live life stages of paropsine beetles (Elek & Wardlaw 2010). To assess adult leaves using a single branch observation technique in New Zealand would require a longer branch length. This is because adult branches undergo rapid internode expansion and 100cm of adult branch is needed to assess browsing damage on multiple nodes. Observations should be timed to occur after at least one generation of *P. charybdis* adult and larval feeding, i.e. around Christmas after feeding by the spring generation or in March following the late summer browsing. If branches were cut down rather than pulled down gently it is common for live insects to fall from the leaves as a fright response, however damage remains. Hence single branch observations are likely to affect the reliability of live insect counts, but the proportion of leaves damaged or missing remains a reliable indicator of recent insect attack.

2. 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 (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 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: Choose a method of sampling each plot, such as a systematic walk following a particular compass direction and assessing every third tree. 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 effected 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: The incidence and severity score for each tree is 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, and decide how to apply the CDI to your individual plantation.

A number of authors have adjusted the CDI method to match their requirements, and its flexibility is one of the reasons it is so useful. For instance Lin et al. (in press) recently undertook assessments of New Zealand Drylands Forest Initiative (NZDFI) young trees of many different species for chewing damage by either *Paropsisterna variicollis* or *Paropsis charybdis*. Lin et al. (in press) used the Step 3 (damage incidence) score but discretised scores into 4 different qualitative categories, and described these (Lin et al. 2017). The method proved useful for making direct comparisons between species and between sites in terms of insect damage observed, however as a qualitative assessment it has limitation for future comparisons.

We wanted to know whether the CDI method would work on older *Eucalyptus* trees as it was designed for assessing younger trees transitioning from juvenile to adult leaf form. We trialled the CDI on two *E. nitens* sites in the Central North Island of New Zealand, between November 2016 and April 2017. The first site was located in Kaingaroa Forest, Compartment 638 (FR509, 5 year old trees), off the Napier-Taupo Road State highway 5 and the second site was located at Poronui Station (20 year old), Taupo.

In the CDI method trial, (Peters 2017) six trees were arbitrarily selected while walking from the edge into the stand were assessed per field site visit using the CDI. Each tree was observed from multiple angles to obtain a clear view of browsing damage on the tree. The crown of the tree was separated into juvenile and mature foliage, and the incidence and severity of insect and fungi damage were calculated separately for each section of the tree. The CDI was calculated by multiplying the incidence and severity divided by 100 for each of adult insect damage, adult fungal damage, juvenile insect damage and juvenile fungal damage. The sum of these incidence/severity products provides the overall tree CDI.

For the CDI to be accurate, the assessor must have a clear view of the entire crown of the tree being assessed. As stated, the CDI method is more suitable for measuring young *Eucalyptus* trees, where canopy closure has yet to occur. The estimation of severity and incidence were much harder to calculate on the larger trees at Poronui as the top of the crown was not clearly visible. Estimates of damage in such trees are approximate at best.

The CDI method requires a large amount of estimation and is based purely on the assessor's opinion. The more familiar the assessor is with understanding the type of damage and what a healthy tree looks like, the more accurate the results will be. Regardless of assessor, the results will always be difficult to standardise due to the large amount of visual estimation required. Despite this, using one assessor for all assessments will reduce measurement bias. Another approach is to use two individuals and then average their independent assessments to minimise observer bias. In our trials the usefulness of the CDI method is severely limited by tree height.

We summarise our findings in the case study below to demonstrate the CDI's usefulness as an assessment method for *E. nitens* trees bearing adult foliage.

3. *Paropsis charybdis* chewing score
This method was trialled on the assessment of *E. nitens* at Kaingaroa Forest, compartment 638, during December 2016 (Suontama & Low 2017). It is relatively quick and simple process, but like the CDI approach it is subjective and may suffer from observer bias.

A ground-based visual assessment from ~ 2 to 4 m was used to score *Paropsis charybdis* chewing in each tree. Trees were scored on a scale from 0 - 5; 0 = no adult foliage to assess, 1 = no *P. charybdis* chewing damage that scaled to 5 = severe *P. charybdis* chewing damage.
Results presented in Fig. 1 show that most 5 year-old *E. nitens* in Kaingaroa Forest had damage (1) or low amounts of chewing damage (2) as at December 2016. Despite this, detailed analysis

(Suontama & Low 2017) indicated a genetic moderate correlation between DBH and chewing (0.56 ±0.04). This suggests that taller trees are actually more prone to *Paropsis* chewing or were assigned higher defoliation scores of the upper crown at age five years. Height at age 5 and length of green crown had very similar estimated genetic correlations (0.55 ±0.03, 0.53 ±0.03) with *P. charybdis* chewing score as DBH. This suggests moderate genetic relationships among these traits (Suontama & Low 2017). There was also a spatial effect (Fig. 2) across the site, which indicates that soil profile or other spatial influences did influence chewing damage scores.

Of concern was the observation that there was a low range of chewing scores within the plot. This leads us to wonder if the full range of palatability of individual trees to *P. charybdis* has been revealed. The majority of trees assessed had no (a score of 1) visible feeding damage (Fig. 1) yet the moderate genetic correlations between chewing score and the length of the green crown, suggest this method was helpful for elucidating differences in susceptibility of seedlots to insect browse.

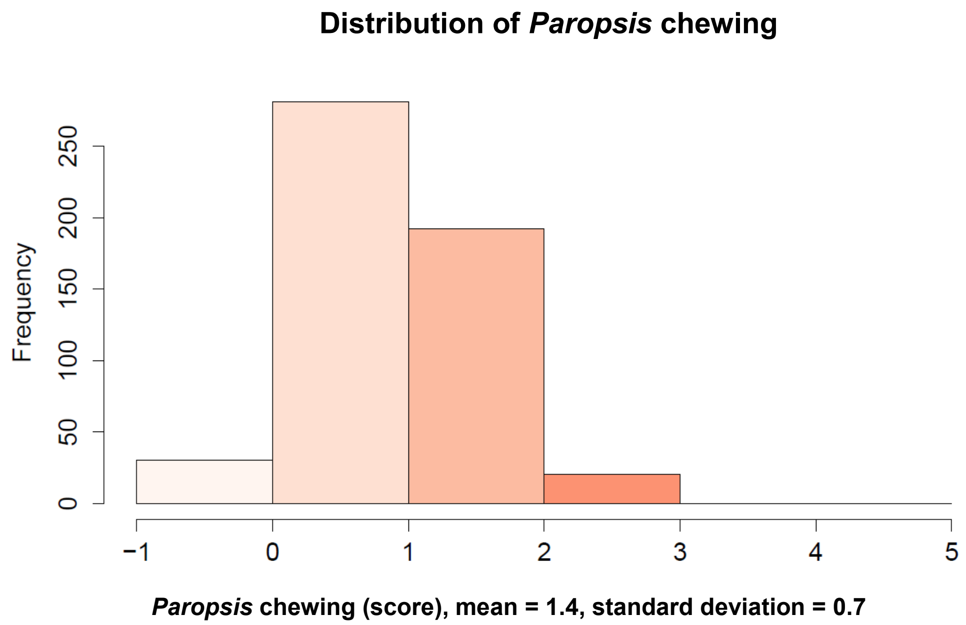

Fig 1. Distribution of *Paropsis* chewing score in FR509, five year old *E. nitens* at Kaingaroa Forest in December 2016. A score of zero means no adult foliage on the tree, one means no damage through to five severe damage.

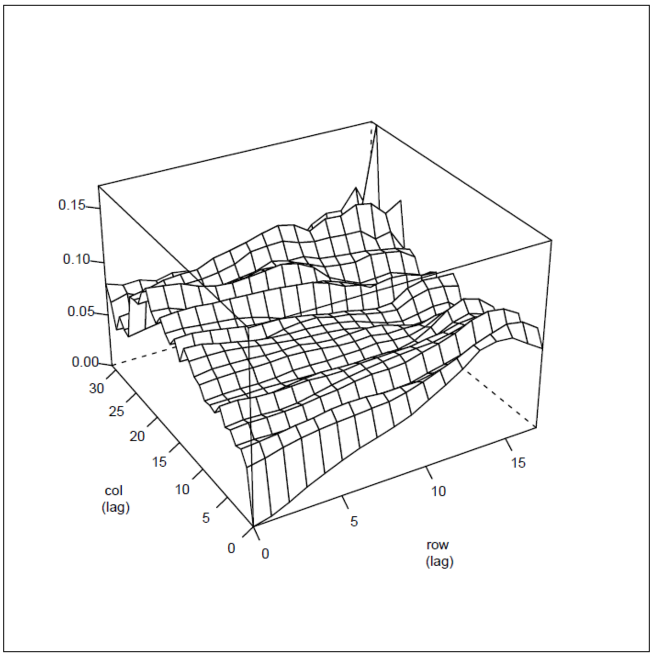

Fig 2. Spatial variation of rows and columns of *Paropsis* chewing score representing spatially the layout of trees within FR509, five year old *E. nitens* at Kaingaroa Forest in December 2016. This figure suggests a spatial relationship may influence chewing damage scores at the site. However, differences are still small.

4. Crown Density score

Another method used to assess *E. globulus* in the Bay of Plenty during 1999 and again on *E. nitens* and *E. fastigata* during 1999-2001 was a visual assessment of crown density (retention of leaves) (Withers et al. 2001). This method is relatively quick and simple to use, but is subjective and has potential to introduce observer bias, just like the previous methods.

Individual trees are observed from two compass directions separated by 90 degrees. A crown density estimate is made using a ten percentile scale ranging from 100% (dense, no leaves missing) through to 10% (virtually completely defoliated, apart from coppice) and the observation is recorded. Assessments are assisted by a set of drawings representing the amount of light/background that can been seen through the crown (Fig. 3). Assessment guides can be accompanied by a set of photographs of trees representative (similar age and same species) of those being assessed. Such guides help minimise observer bias. Having a viable example of a “perfectly healthy” tree at the same age is extremely useful, as eucalypts naturally shed their lower branches as light suppression occurs with canopy closure, and hence the assessment of crown density needs to be in proportion to what could be expected in an ideal situation with no pest or pathogen presence.

Toussaint and Dick (2004) analysed some of the data gathered (Withers et al. 2001) and found that the crown density score was most strongly correlated with diameter at breast height (DBH) (which is also correlated to annual height increment). This correlation was strong across all sites and over two years of monitoring (p=0.0001). Note the causal agent responsible for any defoliation was not recorded with this measure alone.

Table 1. Correlation between DBH increment and categories of crown retention across four sites in the Bay of Plenty, 1999-2001 *E. nitens* planted in 1997 (Toussaint & Dick 2004).

Crown retention	DBH increment year to 2000 (cm)	Probability	DBH increment year to 2001 (cm)	Probability
0-50%	1.37	<0.0001	1.09	<0.0001
50-65%	2.31	<0.0001	1.72	<0.0001
65-75%	2.59	<0.0001	2.62	<0.0001
75-100%	2.92	<0.0001	-	-

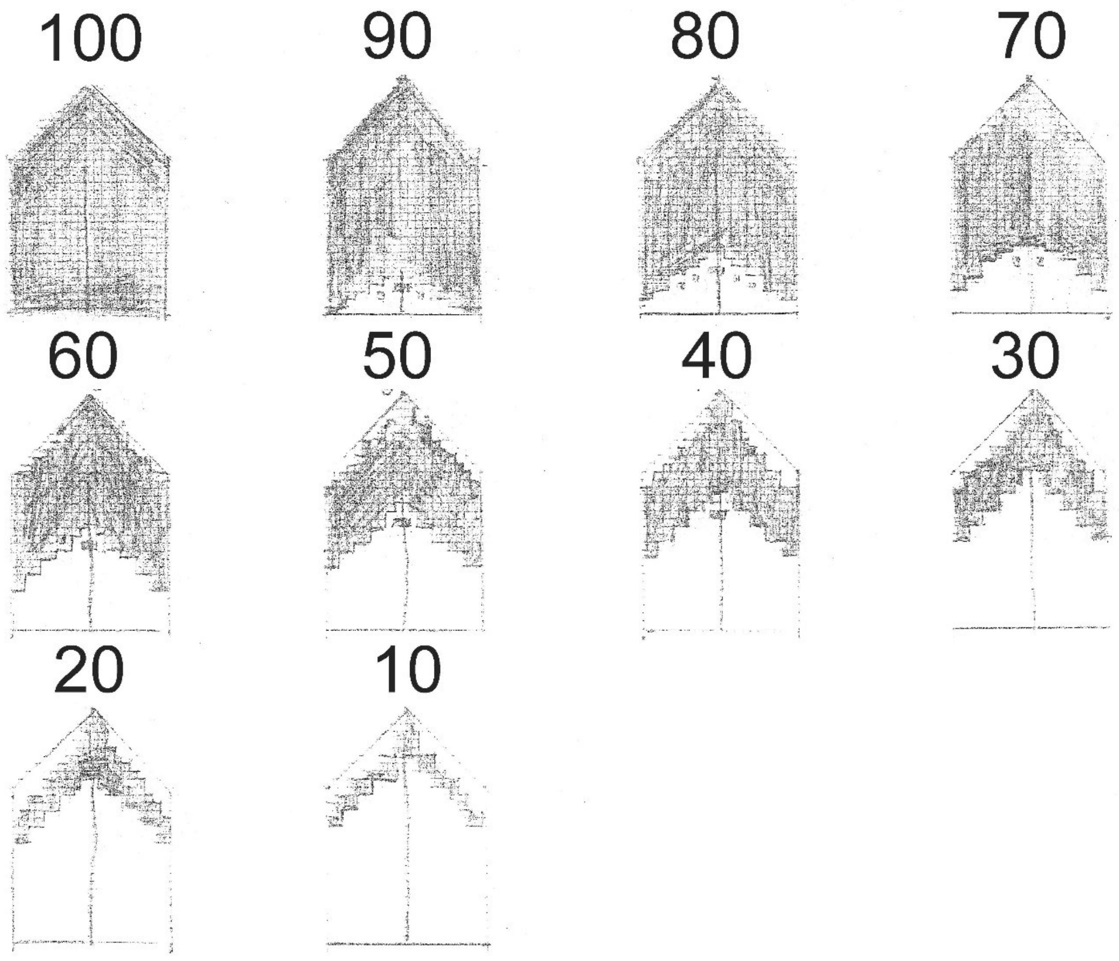

Fig. 3. Set of diagrams (Withers et al. 2001) depicting the % crown density score, a measure of leaf retention in young *Eucalyptus* trees, which has been shown to be a high significant positive correlation to incremental growth of trees between three and five years old (Toussaint & Dick 2004)

RESULTS

Case study: Kaingaroa FR509 vs. Poronui Station

The Crown Damage Index (CDI), along with the presence of each *Paropsis charybdis* life stage and egg parasitism by *Enoggera nassaui, Baeoanusia albifunicle*, and *Neopolycystus insectifurax* was monitored in two *Eucalyptus nitens* forests in the Central North Island of New Zealand between November 2016 and April 2017 (Peters 2017). The first site was located in Kaingaroa Forest, Compartment 638 (FR509), off the Napier-Taupo Road, State Highway 5 (GPS NZTM 1883983E 5697119N). The second site was located at Poronui Station, Taupo (GPS NZTM 1885166E 5672807N). The Kaingaroa site was visited once a week. The Poronui site was visited fortnightly, except the fortnights beginning December 26th and January 9th. Aerial spraying with an insecticide with the active ingredient alpha-cypermethrin was applied to the forest on the 17th and 20th of December 2016 and the 14th of January 2017 at approx. 300 ml/ha. Due to health and safety, Poronui Station was not visited at this time. Trees at Kaingaroa were 5 years of age, while the trees at Poronui were approximately 20 year of age at the time of assessment. Both sites were known to have *P. charybdis* populations present.

The Crown Damage Index (CDI) was used to record and measure the appearance and health of the *Eucalyptus* trees over the season. Six trees were randomly selected to be assessed using the CDI per field site visit by walking from the edge of the plantation inwards at a diagonal. Each tree was observed from various angles to obtain a clear idea of the damage on the tree. The crown of the tree was separated into juvenile and mature foliage for each rating, and the incidence and severity for insect and fungi damage were calculated separately for both sections of the tree. The CDI was calculated by multiplying the incidence and severity divided by 100 for each of adult insect damage, adult fungal damage, juvenile insect damage and juvenile fungal damage. The sum of these incidence/severity products gives the overall tree CDI (Fig 4).

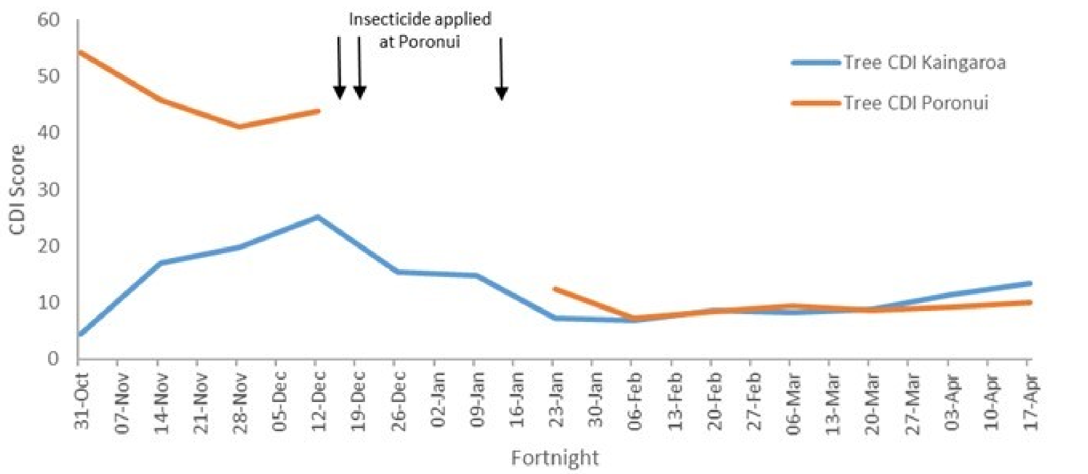

Figure 4. Overall Tree CDI Score at Kaingaroa Forest and Poronui Station per fortnight during 2016-2017 season.

The tree CDI scores change gradually over the first six visits. It is likely this is directly attributable to increasing user familiarisation with the scoring method. The supervising scientist undertook the first two assessments on her own, and then trained the other staff member during the third and fourth visit, and then from the fifth visit the junior staff member became wholly responsible for the scoring.

The juvenile foliage CDI score at Kaingaroa was larger (i.e. damage was worse) than the adult foliage CDI score. Kaingaroa juvenile foliage CDI score was worst during November-December (CDI of 23), while the adult CDI score was lower and consistent across the whole season (Figure 5). The adult foliage CDI score at Poronui was larger (i.e. worse) than the juvenile foliage CDI score. Poronui juvenile and adult foliage CDI scores were worst during November-December, both scores improving after the insecticide spraying late December early January (Figure 5).

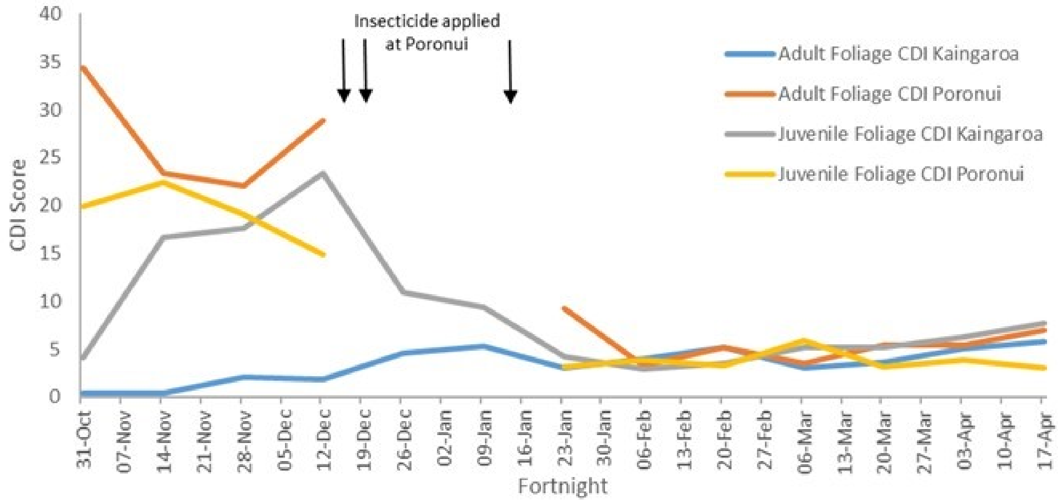

Figure 5. Individual adult and juvenile foliage CDI Scores at Kaingaroa Forest and Poronui Station per fortnight during 2016-2017 season.

Our conclusion from conducting this case study is that population density of *P. charybdis* at Kaingaroa appeared not to be large enough to have any severe visual or any noticeable impact on the *Eucalyptus* trees (Fig. 6). While the overall tree CDI score was worse during the first, spring generation, further analysis showed that it was the juvenile foliage CDI score (Fig. 5, grey line) that contributed to this increase rather than the adult foliage CDI score, which remained relatively constant across the season. This showed that the *P. charybdis* browsing across the season was relatively low (for instance seldom more than 20% of leaf area eaten), and the change in the CDI was due rather to fungal damage on the last remnant of juvenile leaves, than from insect damage. The average CDI adult foliage score at Kaingaroa Forest remained between 0 and 5% across the season, reflecting the relative lack of *P. charybdis* damage in the upper crown (Fig. 5, blue line). The last point shows the CDI adult foliage score assessment undertaken on six different trees each fortnight at Kaingaroa Forest produced very similar results to the *Paropsis* chewing score assessment undertaken on all the same trees only once in December.

Figure 6. Kaingaroa Forest *Eucalyptus nitens* plantation November 2016.

In contrast to Kaingaroa Forest, significant differences in overall tree CDI scores were assessed at Poronui Station (Fig. 4). The spraying of the aerial insecticide between mid-December and early January rapidly reduced the *P. charybdis* population (Peters 2017) which was observed from January as a reduction in overall tree CDI score and specifically adult foliage CDI score. This reflects an improvement in leaf retention, and the presence of flush adult growth that was observed as the return of red colouration to the upper crown of the trees. Adult and juvenile foliage CDI scores at Poronui Station appeared to contribute equally to the overall CDI score of the older *Eucalyptus* trees (Fig. 5). It was observed that after aerial insecticide application the *P. charybdis* population density was reduced, particularly the adults from the first generation. Dead adult beetle bodies were observed littering the forest floor beneath the trees. The aerial spraying allowed the trees to recover (Fig. 8), and produce a new flush of adult foliage, which was reflected in the recorded decrease in the overall tree CDI score as well as a decrease in the adult foliage CDI score (Fig. 5, orange line). However, despite this, a few adults that survived spraying or dispersed into the plantations from unsprayed areas were able to rapidly begin feeding on the flushing *E. nitens* foliage and resume reproduction (Peters 2017). For this reason we expect Poronui Station to retain moderately high levels of *P. charybdis* in the future, and provide a useful site for collecting adults should they be needed for experimental purposes or for supplementing populations at Howdens Block.

Figure 7. Poronui Station *E. nitens* plantation November 2016, prior to spraying.

Figure 8. Poronui Station *E. nitens* January 2017, after spraying, note presence of red coloured flush foliage.

Lessons from the case study

1. Pest population levels. When comparing the two sites and undertaking the assessments that we did, we believe that higher pest levels than were present at Kaingaroa Forest over the 2016/17 season are required before tree level impacts become more strongly indicative of palatability. *E. nitens* has a high threshold for coping with one-off defoliation effects, but if repeated over multiple seasons, particularly when late in the season, we know that economically significant growth suppression occurs (Elek & Wardlaw 2010). Trees that receive any late season defoliation for two consecutive years will be at least 17% smaller in diameter, and mean annual increment (MAI) in diameter will reduce by at least 21% compared with undefoliated trees over one rotation. A higher level of *Paropsis* than the level of *Paropsis* damage (Suontama & Low 2017) present at the Kaingaroa progeny trial (FR509) is necessary to quantify genetic differences in palatability (or resistance). in the NZ breeding population of *E. nitens*. Without sufficient populations of beetles to have fed on all trees, it is a potential waste of resources to undertake an intensive monitoring assessment of Howdens Block.

2. Timing of assessments. Damage correlates to the presence of both adult *P. charybdis* beetles undertaking maturation feeding, and their larvae undergoing development feeding. Adults live longer and therefore eat much more than larvae over time. The most damage and highest adult beetle numbers were recorded at Poronui Station during the outbreak in November and again after recovery from spraying in March, but at Kaingaroa the most larvae were seen in December and adults during February (but these were never high) (Peters 2017). Overall it would be expected that the greatest damage will be observable by the month of March in an unmanaged situation, as it represents cumulative damage from both spring and summer populations.

3. Effectiveness of spray applications. The aerial spray applications of alpha cypermethrin (Withers et al. 2013; Rolando et al. 2016) were initially effective at reducing adult beetle populations at Poronui Station. For unknown reasons sufficient adult *P. charybdis* persisted or re-invaded and resumed reproduction within a month of the spray operation being completed. This may be because each planted area was sprayed on a different date, permitting some highly mobile adult beetles to move between areas and thereby some escaped all the spraying. Biological control agents (egg parasitoids) appeared soon after spraying and controlled a high proportion of egg batches laid in the summer generation (Peters 2017), which was a pleasing result considering many micro hymenoptera are highly susceptible to synthetic pyrethroid insecticides (Withers et al. 2013). This suggests a well-timed spray application can be effective at reducing *P. charybdis* problematic populations without devastating biological control agent populations.

CONCLUSION

As not all methods were trialled in Kaingaroa Forest this year, our recommendations are a combination of what is practical, and what might be ideal. For instance the 10-100% of upper crown density percentile method would be preferable in a plantation suffering a combination of fungal and insect damage. But with the low levels of leaf spot pathogens being experienced at Howden's block, the *Paropsis* chewing score (which measures on a 1-5 scale) should be sufficient to capture the defoliated-state of the crown.

Based on our field trials and a consideration of scientific literature, the following approach is recommended to assess differences in the genetic palatability between seedlots of the *E. nitens* breeding population at Howden's block in Southland. We have taken into account the challenges of assessing *E. nitens* trees over six years old that can be over 12m in height, and that *P. charybdis* attacks adult flush foliage rather than juvenile, and that background population levels in this relatively new plantation is expected to be low.

Recommended steps:

1. <u>Supplementation of pest population</u>. In order to ensure the crown density score of defoliation is greater than 1 (none) out of 5 (as it was at FR509), it is imperative we supplement background populations of *P. charybdis* at Howdens block. The aim would be to achieve an average defoliation of 2 or 3 out of 5 across all *E. nitens* seedlots and feel certain all trees have been exposed to insect browsing. This could be achieved by a transferring a pest beetles from an early spring (October-November – timed to the start of flush at Southland) mass collection of overwintering adult *P. charybdis* beetles from bark and leaf shelters at Poronui Station. At Howdens block a minimum of one live pair of adult beetles should be released per four or five trees at Howdens. This should occur approximately three months prior to assessment and only once flush foliage has begun to appear. This will provide sufficient time for the approximately 2000 adult beetles to undertake maturation feeding, mate and lay at least one hundred eggs per female, and the trees will show the results of both adult (which are highly mobile and likely to move from tree to tree) and larval feeding (larvae are not highly mobile and feed on the tree that the female has chosen to lay her eggs on). Therefore, we would be assessing damage relating to between-tree palatability to adult beetle feeding, as well as their egg-laying preference. There is no data on whether *P. charybdis* adults do choose to lay egg batches on trees which they show a feeding preference for, but this is a common occurrence in many insect pests and is a reasonable assumption to make. After assessments are complete, pest populations may need to be aerially sprayed once or twice, to reduce future impacts on the plantation from the artificially raised beetle population.

2. <u>Quantification of leaves damaged or missing</u>. We consider a visual rating of crown retention alone is insufficient to prove the causal agent is *P. charybdis*. Therefore there are significant benefits to close-up observations of a sample branch from each individual tree. To obtain a branch from such tall trees will be challenging. We suggest that when staff are assessing progeny trial growth traits including defoliation of Howdens Block they attempt to cut down one branch bearing adult foliage. This may be possible by climbing the tree slightly such as with a step (Pro-Lifta-Metre Step or similar, e.g. Lakewood Products Ltd P360, $80) then using extended electric pole pruners (e.g. Lakewood Products Ltd, Arvipo Electronic Trimming Shear with Patented Cutting Head cuts up to 40mm diameter attached to a PS200 2.0m extender gives a total reach of 4 metres, $3500) to obtain one random branch sample bearing adult foliage for quantification of the damage to leaves on the terminal end 100cm (leaf branch score). The staff member on the ground then picks up the branch and assesses both the incidence and severity of feeding damage to adult leaves and quantify those missing completely from each node that should have current season adult expanding leaves present. For instance follow the method to that described in the CDI manual (Stone et al. 2003) to obtain an overall defoliation score for each branch. This is

achieved by scoring the percentage of leaf chewed SEVERITY score according to the figure on page 6 of (Stone et al. 2003) multiplied by a percentage of leaves per node affected by chewing INCIDENCE score. It is expected that some live insects will fall from the branch as it is brought down, but any remaining should be photographed for later identification and counted as further evidence of pest identification and presence. The presence of live insects adds weight to the assumption that the chewing damage is attributable to that particular species, and not to another species that had occupied that same branch prior.

3. Paropsis chewing score. While the individual branch score is being calculated on the branch also undertake a visual rating of each tree crown, being 1-5 of the amount of chewing observable in the trees upper crown (Suontama & Low 2017). This rating has proven to be an adequate measure of crown health, and staff are already familiar with it. Analysis should have a spatial component in order to take into account edge trees, which often experience different growth due to higher light interception.

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