



Technical Report

Report 1: *Eucalyptus* resistance to paropsine beetles

Author/s: Leslie Mann & Stephen Pawson

Date: December 2021

Report: SWP-T140



TABLE OF CONTENTS

INTRODUCTION3Paropsine beetles (Paropsis charybdis (Stål) and Paropsisterna cloelia (Stål))3Identifying genotypes that are resistant or tolerant to defoliation3METHODS5Crown Damage Index (CDI)5Measures of tree growth6Statistical analysis6RESULTS AND DISCUSSION7Species Trial7Eucalyptus bosistoana 2012 family trial13Eucalyptus bosistoana 2018 clones17Eucalyptus tricarpa 2017 families21CONCLUSION25ACKNOWLEDGEMENTS26Species trial26Species trial26Eucalyptus bosistoana 2012 family trial28Eucalyptus bosistoana 2012 family trial23	EXECUTIVE SUMMARY	1
Paropsine beetles (Paropsis charybdis (Stål) and Paropsisterna cloelia (Stål))3Identifying genotypes that are resistant or tolerant to defoliation3METHODS5Crown Damage Index (CDI)5Measures of tree growth6Statistical analysis6RESULTS AND DISCUSSION7Species Trial7Eucalyptus bosistoana 2012 family trial13Eucalyptus bosistoana 2018 clones17Eucalyptus tricarpa 2017 families21CONCLUSION25ACKNOWLEDGEMENTS25Appendix26Species trial26Eucalyptus bosistoana 2012 family trial28Eucalyptus bosistoana 2012 family trial28Eucalyptus bosistoana 2012 family trial28Eucalyptus bosistoana 2012 family trial26Species trial26Species trial26Species trial26Species trial26Species trial26Species trial26Species trial26Species trial28Eucalyptus bosistoana 2018 clonal trial32		
METHODS5Crown Damage Index (CDI)5Measures of tree growth6Statistical analysis6RESULTS AND DISCUSSION7Species Trial7Eucalyptus bosistoana 2012 family trial13Eucalyptus bosistoana 2018 clones17Eucalyptus tricarpa 2017 families21CONCLUSION25ACKNOWLEDGEMENTS25Appendix26Species trial26Eucalyptus bosistoana 2012 family trial28Eucalyptus bosistoana 2012 family trial23		
METHODS5Crown Damage Index (CDI)5Measures of tree growth6Statistical analysis6RESULTS AND DISCUSSION7Species Trial7Eucalyptus bosistoana 2012 family trial13Eucalyptus bosistoana 2018 clones17Eucalyptus tricarpa 2017 families21CONCLUSION25ACKNOWLEDGEMENTS25Appendix26Species trial26Eucalyptus bosistoana 2012 family trial28Eucalyptus bosistoana 2012 family trial23	Identifying genotypes that are resistant or tolerant to defoliation	3
Crown Damage Index (CDI)5Measures of tree growth6Statistical analysis6RESULTS AND DISCUSSION7Species Trial7Eucalyptus bosistoana 2012 family trial13Eucalyptus bosistoana 2018 clones17Eucalyptus tricarpa 2017 families21CONCLUSION25ACKNOWLEDGEMENTS25Appendix26Species trial26Eucalyptus bosistoana 2012 family trial28Eucalyptus bosistoana 2018 clonal trial32		
Measures of tree growth6Statistical analysis6RESULTS AND DISCUSSION7Species Trial7Eucalyptus bosistoana 2012 family trial13Eucalyptus bosistoana 2018 clones17Eucalyptus tricarpa 2017 families21CONCLUSION25ACKNOWLEDGEMENTS25Appendix26Species trial26Eucalyptus bosistoana 2012 family trial28Eucalyptus bosistoana 2018 clonel trial32		
Statistical analysis6RESULTS AND DISCUSSION7Species Trial7Eucalyptus bosistoana 2012 family trial13Eucalyptus bosistoana 2018 clones17Eucalyptus tricarpa 2017 families21CONCLUSION25ACKNOWLEDGEMENTS25Appendix26Species trial26Eucalyptus bosistoana 2012 family trial28Eucalyptus bosistoana 2018 clonal trial32		
RESULTS AND DISCUSSION7Species Trial7Eucalyptus bosistoana 2012 family trial13Eucalyptus bosistoana 2018 clones17Eucalyptus tricarpa 2017 families21CONCLUSION25ACKNOWLEDGEMENTS25Appendix26Species trial26Eucalyptus bosistoana 2012 family trial28Eucalyptus bosistoana 2018 clonal trial32		
Species Trial7Eucalyptus bosistoana 2012 family trial13Eucalyptus bosistoana 2018 clones17Eucalyptus tricarpa 2017 families21CONCLUSION25ACKNOWLEDGEMENTS25Appendix26Species trial26Eucalyptus bosistoana 2012 family trial28Eucalyptus bosistoana 2018 clonal trial32		
Eucalyptus bosistoana 2012 family trial.13Eucalyptus bosistoana 2018 clones17Eucalyptus tricarpa 2017 families21CONCLUSION25ACKNOWLEDGEMENTS25Appendix26Species trial26Eucalyptus bosistoana 2012 family trial.28Eucalyptus bosistoana 2018 clonal trial.32		
Eucalyptus bosistoana 2018 clones17Eucalyptus tricarpa 2017 families21CONCLUSION25ACKNOWLEDGEMENTS25Appendix26Species trial26Eucalyptus bosistoana 2012 family trial28Eucalyptus bosistoana 2018 clonal trial32		
Eucalyptus tricarpa 2017 families21CONCLUSION25ACKNOWLEDGEMENTS25Appendix26Species trial26Eucalyptus bosistoana 2012 family trial28Eucalyptus bosistoana 2018 clonal trial32	Eucalyptus bosistoana 2018 clones	17
CONCLUSION25ACKNOWLEDGEMENTS25Appendix26Species trial26 <i>Eucalyptus bosistoana</i> 2012 family trial28 <i>Eucalyptus bosistoana</i> 2018 clonal trial32		
ACKNOWLEDGEMENTS		
Species trial26Eucalyptus bosistoana 2012 family trial28Eucalyptus bosistoana 2018 clonal trial32		
Species trial26Eucalyptus bosistoana 2012 family trial28Eucalyptus bosistoana 2018 clonal trial32	Appendix	26
Eucalyptus bosistoana 2018 clonal trial		
Eucalyptus bosistoana 2018 clonal trial		
	REFERENCES	

Disclaimer

This report has been prepared by the University of Canterbury for Forest Growers Research Ltd (FGR) subject to the terms and conditions of a research services agreement dated 1 July 2015.

The opinions and information provided in this report have been provided in good faith and on the basis that every endeavour has been made to be accurate and not misleading and to exercise reasonable care, skill and judgement in providing such opinions and information.

Under the terms of the Services Agreement, the University of Canterbury's liability to FGR in relation to the services provided to produce this report is limited to the value of those services. Neither University of Canterbury nor any of its employees, contractors, agents or other persons acting on its behalf or under its control accept any responsibility to any person or organisation in respect of any information or opinion provided in this report in excess of that amount.



EXECUTIVE SUMMARY

A wide range of insect herbivores infest *Eucalyptus* trees in New Zealand, particularly insects native to Australia. The most damaging defoliators are the paropsine beetles. Paropsis charybdis and Paropsisterna cloelia (EVB). In 2019, EVB was present in the North Island, however in the summer of 2019/2020 EVB spread to the upper South Island and now causes considerable defoliation to *Eucalyptus*. Few studies have examined *Eucalyptus* tolerance and resistance to insect defoliation. Understanding paropsine feeding preferences and their impacts is important to inform the selection of New Zealand Dryland Forest Initiative (NZDFI) breeding lines to establish a healthy, productive and durable *Eucalyptus* timber industry in New Zealand.

This research aims to determine how paropsine insects interact with plantation eucalypts in New Zealand. This was achieved by quantifying the resistance and/or tolerance of *Eucalyptus* species/families/genotypes to paropsine attack. Paropsine defoliation across seven different Eucalyptus species (variation at species level), E. bosistoana and E. tricarpa families (variation at family level), and *E. bosistoana* clones of different genotypes (variation at genotype level) were quantified. For each tree sampled, the number and length of the new growth shoots, height increment, DBH increment, and defoliation using the CDI (Crown Damage Index) were assessed to quantify defoliation and resistance/tolerance on two or three occasions between 2019-2021. CDI was used to measure resistance, whereas growth measurements (DBH, height and new shoot growth) were used to assess tolerance to paropsine browse.

Key results are:

- 1. At the species level: E. cladocalyx, E macrorhyncha and E. globoidea were the most resilient to defoliation, whereas E. guadrangulata and E. tricarpa were the least resilient. E. globoidea and E. cladocalyx were the species with the highest growth rate (height and DBH increment).
- 2. Large variation in defoliation was recorded between and within *E. bosistoana* families. There was a weak negative correlation between CDI and height growth but no correlation between CDI and DBH growth for E. bosistoana families. E. bosistoana family 805 was constantly resistant to defoliation across all measurements periods and recorded a good growth rate (height and DBH increment). All E. tricarpa families were heavily defoliated with minimal variation in browse observed between families.
- 3. Substantial variation in the CDI and growth was observed within the same E. bosistoana genotype (i.e., individuals that were genetically identical). This indicates that other microsite factors are important influencers of defoliation and growth.
- 4. Multiple comparison tests were not possible for clonal and family trials due to the large number of potential comparisons. Thus, tables containing the 10 best and 10 worst families for CDI, height and DBH increment are provided for the *E. bosistoana* family and clonal trials and the *E. tricarpa* family trial.

Recommendations:

- 1. All E. tricarpa families were highly defoliated and only moderate height gain was observed. E. guadrangulata was extremely defoliated and attacked by other insect pests as well. From a pest management perspective, these two species have low resistance to paropsines and continued development of these species should be reconsidered.
- 2. E. globoidea and E. cladocalyx and E. macrorhyncha seem to be promising species that are resistant to paropsine browse, and maintain strong growth rates (DBH and height). Moreover, some individual *E. globoidea* trees seem to be more resistant and/or tolerant. E. bosistoana families expressed variable resistance to paropsine defoliation.

They had some growth rate differences (height and DBH increment) as well that were not necessarily negatively correlated with paropsine resistance, leading to some tolerance abilities. However, questions remain regarding the influence of microsite factors. We recommend planting such individuals in controlled trials using homogenous conditions (soil, moisture, slope etc.) to assess the heritability of the observed resistance/tolerance in the absence of potentially confounding microsite factors.

INTRODUCTION

Paropsine beetles (Paropsis charybdis (Stål) and Paropsisterna cloelia (Stål))

The tribe Paropsini (paropsine leaf beetles) has over 400 species that feed almost exclusively on eucalypts. Paropsine beetles are commonly called tortoise beetles because of their turtle shape. They can have many different phenotypes, and most species are native to Australia (Jolivet, Cox, & Petitpierre, 1994). In total, six paropsine species have established in New Zealand (Withers, 2001) (Paropsis charybdis, Trachymela sloanei (Blackburn), Trachymela catenata (Chapuis), Trachymela sp., Paropsisterna cloelia and Paropsisterna beata (Newman)). Each species has different impacts on Eucalyptus trees. P. charybdis was first detected in New Zealand in 1916, and spread quickly due to a lack of natural enemies and many host species available in the subgenus Symphyomyrtus (Withers & Peters, 2017). Paropsisterna cloelia established in the Hawke's Bay (North Island) in 2016 and has dispersed to the South Island in 2018-2019. Lin et al. (2017) found that the newly established Pst. cloelia was more abundant and voracious than P. charybdis. This was tentatively attributed to the longer activity of *P. cloelia* through the year compared to *P. charybdis*. Although *P. charybdis* and Pst. cloelia eggs, larvae and adults can be easily segregated, their feeding damage is indistinguishable.

Paropsine attraction to different eucalypt species is influenced by foliage density, nutritional state of the leaves, and the presence of defensive compounds. Trees with dense, young, foliage are more attractive to defoliators (Jolivet et al., 1994). In addition, weather conditions, soil composition, nitrogen leaf availability and leaf toughness all moderate the phenology and abundance of paropsine beetles. Eucalyptus growing in non-fertile soils tend to have more defensive compounds than those living in fertile areas (Jolivet et al., 1994). It is hypothesized that this difference occurs because trees in poor soil need to defend themselves against predators as they have limited capacity to grow new foliage in response to herbivory, hence chemical defenses are used to repel herbivores (Stone, 2001). Conversely, trees growing in fertile areas can compensate for herbivory by growing new shoots in response to defoliation. However, when plants are stressed, they can become more nutritious with higher nitrogen and carbohydrates content due to a high tolerance. This is more attractive to herbivores and is one mechanism by which herbivore outbreaks occurs (White, 1984). Different methods have been used to quantify herbivory on *Eucalyptus* genus. The most common methods are visual ground assessments, e.g., Crown Damage Index (Stone et al., 2003). Other methods using growth rate can be employed as well (dos Santos Bobadilha et al., 2019; Karen J. Marsh, 2019; O'Reilly-Wapstra, McArthur, & Potts, 2004).

Identifying genotypes that are resistant or tolerant to defoliation

The NZDFI breeding program wants to develop trees with improved growth rate and wood properties. To achieve this, they must consider pest resistance/tolerance as defoliation by paropsines will impact growth. Species, families or provenances are considered resistant to herbivory if insects do not feed, or feed only as a last resort. Conversely, trees are tolerant if they have adapted to this defoliation and recover from defoliation with minimal growth loss. Differences in resistance could be explained by variation in the chemical compounds present in the leaves. If a tree is highly toxic, not many insects will feed on it, even the specialized ones. Tolerance can be explained by higher nitrogen and carbohydrates stock, and a higher photosynthetic rate, which provides the ability to grow new leaves in response to defoliation. Specialized insects can adapt to overcome the defences that make a tree species or family resistant. Alternatively, tree species and families can adapt to herbivory. This is an evolutionary "arms race" with perpetual selection pressures on attributes of the insect and the



tree. Resistance is about reducing herbivory attack itself. Tolerance is more about limiting the negative effects of defoliation without attempting to prevent it. Hence, tolerant species/individuals retain good growth rate despite herbivore browse (Restif & Koella, 2004). Tolerance as a strategy presents a potentially more stable coevolution in comparison to resistance (De Jong & Van Der Meijden, 2000; Leimu & Koricheva, 2006). However, these two defence mechanisms are not mutually exclusive and often co-occur (Leimu & Koricheva, 2006; Restif & Koella, 2004). Tolerance and resistance should be studied concurrently to evaluate which strategies are used by Eucalyptus to support decisions of the breeding programme.

Susceptibility to insect herbivory varies between and within a species. Huimin Lin (2017) assessed resistance of different Eucalyptus species and E. bosistoana families in the Hawke's Bay. She showed that E. cladocalyx, E. macrorhyncha and E. globoidea have the lowest levels of herbivory amongst eleven species assessed (Figure 1). Our study follows from Huimin Lin's work and provides a broader assessment of resistance of the NZDFI species that also verifies the consistency of resistance amongst different sites. Trees with no or light defoliation are considered as resistant. Conversely, trees with moderate to severe defoliation level, but which show a high recovery/growth rate following defoliation, are considered as tolerant. Lin (2017) measured the refoliation of 14 E. bosistoana families and observed large variation, especially in response to moderate defoliation, and a higher negative health impact when the defoliation occurred in late spring and/or several times during the year. However, she did not measure differences in recovery between Eucalyptus species or between families other than E. bosistoana. To fill this gap. I conduct experiments to evaluate the resistance and tolerance of seven Eucalyptus species, 74 families of E. bosistoana and 16 E. tricarpa families, and variation amongst genotypes (clones) of E. bosistoana and E. tricarpa that incorporate measures of growth in response to herbivory.

In this study, resistance and tolerance were assessed at three different levels: 1) Evaluating differences between 7 Eucalyptus species, 2) assessing 74 E. bosistoana families and 16 E. tricarpa families, and 3) assessing variation amongst clones of specific E. bosistoana genotypes

METHODS

Four field-based trials of resistance/tolerance were assessed:

- Defoliation assessment of the 7 Eucalyptus species (E. quadrangulata, E. bosistoana, E tricarpa, E. globoidea, E. macrorhyncha, E. camaldulensis and E. cladocalyx) at the Dillon and Lissaman trial sites. These trees were planted in 2011 at Dillon and 2013 at Lissaman to assess wood quality. Fifteen trees were assessed per species at each site.
- 2. The 74 *E. bosistoana* families at Dillon (2012 planting) were assessed by sampling three trees per family.
- The 2018 planting of *E. bosistoana* clones at Dillon was assessed. Clones give results with naturally low noise level by eliminating genetic variability factors (Simon P. Whittock, 2003). Thus, clones from the same genotype should not respond differently to herbivory. In total 621 *E. bosistoana* clones were assessed from 132 genotypes belonging to 73 families.
- 4. Three trees from each of the sixteen *E. tricarpa* families were assessed from each of the 2017 plantings at Dillon and Lissaman.

Fieldwork occurred during the summer season when paropsine beetles are active. Three assessments were conducted in December 2019/January 2020, October 2020, and March 2021. In October, only overwintering larvae and adults are present but no new egg batches have yet been laid. December represents the greatest activity period of paropsines with eggs, larvae and adult present. March is the end of the summer season where mostly only adults are found in the field.

Crown Damage Index (CDI)

The CDI method is a visual defoliation estimate of the entire tree. A derivative of the CDI is the CDI shoot assessment that evaluates three shoots that are observed in detail as a substitution for a full tree crown assessment. This is more practical with taller trees where a pole pruner allows sampling of upper crown shoots where paropsine beetle damage occurs. The CDI score is calculated as the (Incidence*Severity)/100. The incidence is the number of damaged leaves per shoot scored as an average from three shoots. The severity is the average level of damage per leaf, again averaged across three shoots (Stone et al., 2003). This defoliation level measurement is the most common method to assess *Eucalyptus* defoliation in Australia (Stone et al., 2003). In the absence of quantitative remote sensing methods, the CDI is a good compromise between precision and time spent assessing trees. CDI estimation is only semi-quantitative and potentially prone to observer bias.

5



Image 1: Example of severity index for paropsine damage, Stone et al., 2003.

Measures of tree growth

Diameter at Breast Height (DBH) was measured at 1.4 m from the ground with a calibrated diameter tape. At first measurement a paint mark was added as a reference for remeasurement. For E. tricarpa, DBH was only assessed at the Lisssaman site, as Dillon trees were too small to measure the DBH. No DBH was recorded at the *E. bosistoana* 2018 clonal trial due to the small size of the trees. Tree height was measured with a vertex or a height pool depending on the tree size. The number of new shoots and the longest length of new shoot were measured on each of the three shoots assessed using the CDI score. At the beginning of the summer season (early October) the new shoots are easily distinguishable (softer, light green) as the season progresses we used the perceptible mark on the stem that indicates the start of the new season growth.

Statistical analysis

R (using R-studio interface) and excel were used for statistical analysis and data collection/preparation, respectively. Most data contained two or more variables to analyse (the species/family/clone, sites, date and with the plot as a random effect in function of the height, DBH, CDI). A linear mixed model using a Satterthwaite's method type III ANOVA and a t-test (ImerModLmerTest) was used for the species trial to compare the CDI, DBH, tree height, length and number of new shoots between species, sites or dates. Finally, simultaneous tests for general linear hypotheses using a multi-comparison Tukey test species was used to assess variation in term of height, DBH and CDI between species.

The Satterthwaite's method's type III ANOVA and a t-test (ImerModLmerTest) was used for the E. bosistoana 2012 and 2018 family trial and for the E. tricarpa 2017 family trial to assess the CDI, DBH, tree height, length and number of new shoots regarding different families, sites or dates. However, a multi-comparison Tukey test was not appropriate to assess family and clonal variations for the E. bosistoana 2012 family and E. bosistoana 2018 clonal trials due to the number of family or clone comparisons. Instead, a multivariate generalised linear mixed model using a Bayesian test was used to process differences between families and clones. A Markov chain Monte Carlo model was then used to create a posterior distribution of 1000 samples to test whether the best family was always significantly different from the worst family. Because only 3 or 6 trees per family were assessed for E. bosistoana 2012 and E. tricarpa and 4 to 12 trees per genotype for the *E. bosistoana* 2018 trial, the estimated means from the model are used. Using estimates of the model rather than the real data provides more stable estimates when sample sizes are small.

RESULTS AND DISCUSSION

Across all trials the CDI varied with time, this reflects the interaction between the spring flush of new leaves and paropsine phenology, as insects are more abundant in summer compared to spring. For all trials the CDI was low in October with higher levels of defoliation in December and March (Table 1).

Species Trial

Resistance

Mean CDI from December 2019 to March 2021 was lower at Lissaman compared to Dillon (Dillon=31.2 \pm 1.34, Lissaman=21.0 \pm 1.18, Satterthwaite's method type III ANOVA, P<0.01). This site effect may be due to the lower density of *Pst. cloelia* that was observed (but not quantified) at Lissaman. It is not known why there was an observable population level difference in paropsines between the two sites.

Table 1: Mean and standard error of defoliation level (CDI) during the three measurement dates (December 2019, October 2020, and March 2021) at Lissaman and Dillon. Mean CDI= mean of Crown Damage Index. Overall Lissaman was less defoliated compared to Dillon (Satterthwaite's method type III ANOVA, P<0.01). In October 2020, trees were less defoliated compared to December 2019 and March 2021 (P<0.01).

Date	Site	Mean CDI
December 2019	Dillon	37.12 ±2.21
	Lissaman	32.77 ±2.19
October 2020	Dillon	11.13 ±1.27
	Lissaman	3.34 ±0.54
March 2021	Dillon	44.69 ±1.93
	Lissaman	26.58 ±1.51

Mean CDI scores differed between eucalypt species (Satterthwaite's method type III ANOVA, P<0.001). *E. cladocalyx* and *E. macrorhyncha* were more resistant to paropsine browse than *E. quadrangulata*, *E. camaldulensis*, *E. tricarpa*, and *E. bosistoana* ((Multicomparison Tukey test, P<0.001, Figure 1, Table 2 and Table A1). *E. globoidea* was more resistant than *E. quadrangulata*, *E. camaldulensis* and *E. tricarpa* (Multicomparison Tukey test, P<0.05 or less, Figure 1, Table 2 and Table A1).

At Lissaman, most species (excluding *E. macrorhyncha* and *E. cladocalyx*) were more defoliated in December 2019 than in March 2021 (Table 1). This could reflect inter-annual variation in climate and hence paropsine abundance and stage of development. In contrast, Dillon, which was visually more infested by paropsines (particularly *Pst. cloelia*) compared to Lissaman) had a higher CDI in 2021 compared to 2019 (Table 1). Contrasting explanations for this is either an increase in EVB density, climatic differences between 2019 and 2021, or alternatively a decrease in the capacity of trees to recover from past defoliation. This latter possibility would manifest by a gradual reduction in the production of new leaves, hence CDI increases as trees retain greater proportions of older damaged leaves per shoot. This is something to continue to assess as *Pst. cloelia* becomes more established in the South Island, i.e., will we observe a gradual decline in tree health from repeated defoliation?

In addition to paropsine damage, *E. quadrangulata* was heavily affected by many other insects, including *Eucalyptus* weevils (*Gonipterus scutellatus*), leaf rollers (*Strepsicrates macropetana*) and sawflies (*Phylacteophaga froggatti*)). Other *Eucalyptus* species were also impacted by these species, but to a lesser extent. Although, no effort was made to quantify

⁷ SWE

herbivory from the full suite of potential pests, our observations highlight that other herbivores are a significant problem for some plantation *Eucalyptus* species.

Tolerance

There were differences in growth between the eucalypt species sampled (Satterthwaite's method type III ANOVA, height increment P=0.001, DBH increment P=0.013). *E. cladocalyx* had a higher height gain compared to *E. quadrangulata and E. bosistoana* (Multi-comparison Tukey test, P=0.023 and P=0.019 respectively, Table 2 and Table A1). *E. globoidea* had greater DBH gain compared to *E. quadrangulata*, *E. camaldulensis*, *E. tricarpa*, and *E. bosistoana* (Multi-comparison Tukey test, P<0.004 or less, Table 2 and Table A1).

In October 2020, there were variations in the length of new shoots grown between species (Satterthwaite's method type III ANOVA and t-test, P<0.001, Table A1). Due to higher resources available (more photosynthesis if more leaves), a less defoliated tree should grow more foliage than a defoliated tree. But the opposite was observed in the field. Trees that had the longest new shoots were also the most defoliated, e.g., in Dillon site, E. guadrangulata had the longest shoots of all species, followed by E. bosistoana, E. camaldulensis and E. tricarpa (Table A1). The strength of the new shoot growth in response to defoliation is potentially a sign of tolerance whereby trees attempt to regrow leaves before the new summer growing season. E. globoidea, E. cladocalyx and E. macrorhyncha had the shortest length of new shoots (Table A1). These less defoliated tree species possibly invest their energy in defensive compounds rather than new growths. Another explanation could be that defoliation could reduce water stress in case of a drought (Igbal, Masood, & Khan, 2012). The summer 2019 was especially dry. It is possible that herbivory may have reduced drought impact for defoliated trees compared to the healthy ones, leading to a bigger growth for defoliated trees. There were no differences in the length of new shoots between species in March 2021 (Satterthwaite's method type III ANOVA, P>0.05, Table A1). It may be due to two different facts: a) some species that had smaller shoots in October are growing faster during the summer, meaning that all species had the same new foliage length in March b) measurement error was higher in March as it was hard to measure the growth length on several species because the growth marks were less obvious later in the year. There was no difference in the number of new shoots grown between the Eucalyptus species sampled (Satterthwaite's method type III ANOVA, P>0.05, Table A1).

Correlation between CDI and growth rate

The relationship between the rate of paropsine browse from March 2021 versus height and DBH increment between October 2020 and March 2021 suggest that paropsines may have a negative impact on the growth rates of some *Eucalyptus* species at Dillon and Lissaman (Figures 2 and 3). On average across all species, there was a strong correlation between overall tree height and DBH in March sampling (Pearson's method, cor=0.902, P<0.001). However, the correlation between the height and DBH increment throughout the sampling period (from October 2020 till March 2021) was weak (cor=0.47, P<0.001, Pearson's method). Correlation between CDI (March 2021, when defoliation was at its maximum) and height/DBH increment was weak or without any correlation respectively (Pearson's method, height cor=-0.41, P<0.001, DBH cor=-0.34, P<0.001, Figures 2 and 3). Analyzing individual species, there was a negative correlation (cor=-0.43, P=0.0238) between CDI score and height increment for *E. tricarpa*.

Low correlation could reflect variation in the tolerance of individual trees within a species to paropsine browse. Importantly variation in defoliation and growth rates was observed between and within species that could provide avenues for the NZDFI breeding program. Some individuals, mainly with a low CDI, seem to grow well (resistant trees, Figures 2 and 3). Individuals from the *E. globoidea* and *E. macrorhyncha* that had both a height increment equal or higher than 1.40 m and a DBH increment equal or higher than 2.5 cm: *E. macrorhyncha* P5T13, P21T13 and P21T33, *E. globoidea* P4T41 and P4T38 (P=plot number, T= tree number

from the species trial) are potential targets for further work. A next step would be to plant clonal trials of these individuals to test the repeatability of observations under more controlled conditions. Currently our results are based on a single individual that might have benefited from specific, unmeasured, microsite factors.

Table 2: Multicomparison (Tukey) test P-values for Crown Damage Index (CDI) score, height and DBH increment from December 2019 to March 2021. Average of three measurement period: December 2019, October 2020 and March 2021. quadr=*E. quadrangulata*, Cama= *E. camaldulensis*, tric= *E. tricarpa*, bosis= *E. bosistoana*, clado= *E. cladocalyx*, macr= *E. macrorhyncha*, glob= *E. globoidea*. The first column species are the species with the highest CDI score and the lowest height and DBH increment. The second column are the species with the lowest CDI score and the highest height and DBH increment. *E. cladocalyx* and *E. macrorhyncha* were most resistant to paropsine browse compared to *E. quadrangulata*, *E. camaldulensis* and *E. tricarpa*. *E. cladocalyx* height increment was higher compared to *E. quadrangulata* and *E. bosistoana*. *E. globoidea* DBH increment was bigger compared to *E. quadrangulata*, *E. camaldulensis*, *B. tricarpa*, and *E. bosistoana*. *E. globoidea* to *E. quadrangulata*, *E. camaldulensis* and *E. tricarpa*.

	CDI score		CDI score Height gain			ain	DBH gain		
Species comparison	P-valu	е	P-valu	е	P-valu	ie			
clado - quadr	< 0.001	***	0.0232	*					
macr - quadr	< 0.001	***	0.0994						
glob - quadr	< 0.001	***			0.00182	**			
clado - cama	< 0.001	***							
macr - cama	< 0.001	***							
glob - cama	0.001	**			0.0024	**			
clado - tric	< 0.001	***	0.0909						
macr - tric	< 0.001	***							
glob - tric	0.004	**			0.00329	**			
clado - bosis	< 0.001	***	0.0189	*					
macro - bosis	< 0.001	***			0.08153				
globo - bosis	0.051				< 0.001	***			

Table 3: Correlation and P-values of 7 eucalyptus species analysed for each of three different correlations: Height increment versus DBH increment, CDI score versus Height increment, CDI score versus DBH increment. Four correlations had a significant P-value: *E. tricarpa* had a moderate positive correlation (0.61) between height and DBH increment and a low negative correlation (-0.43) between CDI score and height increment, *E. globoidea* had a moderate positive correlation (0.58) between height and DBH increment, *E. macrorhyncha* had a high positive correlation (0.78) between height and DBH gain.

Species	Height gain/DBH gain			CDI/Height gain			CDI/DBH gain	
	cor.	P-value		cor.	P-value		cor.	P-value
E. quadrangulata	0.44	0.18		-0.16	0.63		0.19	0.58
E. cladocalyx	0.19	0.34		0.28	0.15		0.19	0.34
E. tricarpa	0.61	< 0.001	***	-0.43	0.0238	*	-0.22	0.27
E. bosistoana	-0.31	0.15		-0.38	0.07		0.26	0.21
E. camaldulensis	-0.13	0.58		-0.16	0.49		0.07	0.76
E. globoidea	0.58	0.0047	**	-0.04	0.86		-0.14	0.53
E. macrorhyncha	0.78	< 0.001	***	-0.15	0.47		-0.28	0.17



Figure 1: Mean Crown Damage Index (CDI) with standard error of the 7 *Eucalyptus* species during the three measurement periods at two sites in the Marlborough region. Sites= Dillon and Lissaman, Measurements= December 2019, October 2020 and March 2021. Cama= *E. camaldulensis*, tric= *E. tricarpa*, bosis= *E. bosistoana*, quadr=*E. quadrangulata*, clado= *E. cladocalyx*, glob= *E. globoidea*, macr= *E. macrorhyncha*. *E. macrorhyncha*, *E. cladocalyx* and *E. globoidea* were the least defoliated. *E. quadrangulata*, *E. camaldulensis*, *E. tricarpa* and *E. bosistoana* were the most defoliated. Mean defoliation at Lissaman was less than Dillon. In October, leaves were less attacked compared to December and March.



Figure 2: Scatterplot of the height increment (m) and Crown damage Index (CDI) score of the 7 species trial during March 2021. Each point represents one individual tree. Each ellipse represents the graph area that each individual species could cover. Points in the top right of the plot represents trees that are tolerant to paropsines, whereas point in the bottom right represents resistant trees. *E. macrorhyncha*, *E. cladocalyx* and *E. globoidea* are more resistant to paropsine damage. *E. quadrangulata* is extremely susceptible. *E. tricarpa* individuals have a wide range of defoliation variation. *E. cladocalyx* individuals have a wide range of height increment variation.



Figure 3: Scatterplot of the Diameter at Breast Height (DBH) increment (cm) and Crown Damage Index (CDI) score of the 7 species trial during March 2021. Each point represents one individual tree. Each ellipse represents the graph area that each individual species could cover. Points in the top right of the plot represents trees that are tolerant to paropsines, whereas point in the bottom right represents resistant trees. *E. macrorbyncha*, *E. cladocalyx* and *E. globoidea* are more resistant to paropsine damage. *E. quadrangulata* is extremely susceptible. *E. tricarpa* and *E. cladocalyx* individuals have a wide range of defoliation variation. *E. globoidea* individuals have a wide range of DBH increment variation.

Eucalyptus bosistoana 2012 family trial

Differences in defoliation, height and DBH gain were observed between families (Satterthwaite's method type III ANOVA, P<0.001 for CDI score, height and DBH increment, Figures 4-5). Statistically it was not possible to conduct a multiple comparison test amongst all families, thus we used a Markov chain Monte Carlo model to test whether the best family was always significantly different from the worst family. The probability that the family with the largest mean score for a particular co-efficient is different from the smallest family was: CDI coefficient probability =1, height coefficient probability =0.997, and DBH coefficient probability =1). We present the 10 best and 10 worst family responses for CDI score, height and DBH increment (Tables 4 and 5) and provide an overview of a) how the families are growing in the field, b) how susceptible families are to paropsine browse. Large variation in paropsine browse within families was observed as indicated by the standard errors.

Defoliation was not consistent over time, only family 805 was consistently placed in the ten least defoliated families in all three sampling periods (Table 4). Similarly, high levels of defoliation amongst families were not consistent (Table 4). The height and DBH increment did not fluctuate through time as much as the observed defoliation (Table 5). Unexpectedly, for a few families, the DBH was smaller in March 2021 compared to October 2020 (Table 5). This could be due to measurement error (despite painted marks being placed on trees to permit consistent placement of measurements), however, alternatively the 2020-2021 summer season was an extremely dry period. Due to this drought, it is possible that the *Eucalyptus* did not grow, and potentially shrank due to the lack of water. Reich and Borchert (1984) have shown that in specific tree species, stem shrinkage can occur after a decline of water potential.

Correlation between CDI and growth rate

A comparison between CDI and growth rate (DBH or height increment) can help us to identify families/individual trees that are resistant or tolerant to paropsine browse. Analyzing all individuals with a correlation test showed no correlation between CDI and height/DBH increment (Pearson's method, height cor=-0.22, P=0.001, DBH cor=-0.086, P=0.24). In addition, there was no correlation between height and DBH increment (Pearson's method, cor=0.245, P<0.001). Correlation tests at the family level were possible but should be viewed with caution as they contain few individuals per family. Family 823 gave a negative correlation between CDI and height increment (cor=-0.999, P-value=0.33, number of trees=3), and the family 879 has a positive correlation between CDI and DBH increment (cor=0.999, P-value=0.027, number of trees=3). Some individual trees had strong tolerance to paropsine browse. Despite a CDI score of over 60 they maintained a height increment of >0.5 meter and DBH increment >0.5 centimetres (individual trees 824a and 859a, Figures 4 and 5). Other individuals showed a good resistance capacity with a low CDI and a high DBH/height increment, e.g., 805a, 861a and 849a (Figures 4 and 5). The family 859 could be a resistant family with a low CDI and a good growth rate (Tables 4 and 5).

Variation within the same family was important, leading to the hypothesis that individual variation occurs. To verify if individual variation in *Eucalyptus bosistoana* occurs, an analysis of the genotype variation with the 2018 clonal trial planting adjacent to the *Eucalyptus bosistoana* 2012 trial could be assessed. The next step after identifying these resistant or tolerant families/individuals will be to grow clones in a homogenous area to avoid microsite factors (soil, water availability, slope, wind exposure, etc.)



Figure 4: Scatterplot of the height increment (m) and Crown Damage Index (CDI) score of E. bosistoana families from the 2012 planted trial during March 2021. Each point represents one individual tree with those in the top right of the plot being most tolerant trees and those in the bottom right being most resistant to paropsine browse. The three first number of each label represent the family number and the letter represents the individual in each family. A few individuals seem to be more resistant to paropsine damage (830a, 811a, 814a, 862a, 809a, 862a, 805a, 849a and 861a). A few individuals seem to be more tolerant to paropsine damage (824a, 819a, 807a, 811a, 859a, 827a, 812a, 811b, 855a, 825a and 809a). Label and localisation: P=plot number, T= tree number. 803a=P10T4, 805a=P76T26, 807a=P10T12, 808a=P10T11, 809a=P9T23, 809b=P29T13, 810a=P30T19, 811a=P9T3, 811b=P29T6, 812a=P76T22, 813b=P29T12, 814a=P75T34, 814b=P30T18, 819a=P29T34, 824a=P10T18, 825b=P76T6, 827a=P10T15, 830a=P9T22, 839a=P30T16, 839b=P76T24, 848a=P10T9, 849a=P76T3, 853a=P75T1, 854a=P10T14, 855a=P29T26, 859b=P29T1, 860a=P30T9, 861a=P75T23, 862a=P10T4, 862b=P30T22, 862c=P76T25, 869a=P30T34, 877a=P30T36, 889a=P29T20, 991a=P29T18, 996a=P75T36.

14



Figure 5: Scatterplot of the Diameter at Breast Height (DBH) increment (cm) and Crown Damage Index (CDI) score of the E. bosistoana family from the 2012 planted trial during March 2021. Each point represents one individual tree with those in the top right of the plot represents tolerant trees. Bottom right represents resistant trees. The three first numbers of label represent the family. The letter represents the individual in each family. A few individuals seem to be more resistant to paropsine damage (830a, 861a, 849a and 805a). A few individuals seem to be more tolerant to paropsine damage (835a, 824a, 846a, 818a and 859a). Label and localisation: P=plot number, T= tree number. 803a=P10T4, 805a=P76T26, 812a=P76T22, 813a=P10T20, 818a=P11T34, 824a=P10T18, 825b=P76T6, 829a=P30T10, 830b=P71T26, 835a=P29T17, 846a=P76T5, 849a=P76T3, 856a=P30T26, 859a=P10T19, 861a=P75T23.

15

Table 4: Mean and standard error of Crown Damage Index (CDI) score of the 10 families with the lowest and 10 with the highest measurement recorded. Families from the *Eucalyptus bosistoana* families trial planted in 2012 in the Marlborough region. Three measurement periods: December 2019, October 2020 and March 2021.

Crown Damage		December		October		
Index (CDI)	Family	2019	Family	2020	Family	March 2021
	991	14.04 ±6.213	830	1.28 ±6.213	996	32.45 ±6.213
	869	15.84 ±6.212	890	1.34 ±6.212	854	33.19 ±6.212
	859	18.19 ±6.212	804	1.63 ±6.214	884	33.88 ±6.218
	846	18.71 ±6.213	859	2.26 ±6.212	819	34.49 ±6.212
Lowest CDI	839	18.99 ±6.212	884	2.35 ±6.218	802	35.14 ±6.212
Lowest CDI	805	19.69 ±6.212	865	2.58 ±6.212	873	35.73 ±5.381
	842	19.98 ±7.606	833	2.84 ±6.212	862	35.91 ±6.212
	855	20.04 ±6.213	801	2.95 ±6.212	801	36.08 ±6.212
	809	20.17 ±6.212	886	3.09 ±6.212	830	36.51 ±6.213
	826	21.33 ±6.212	805	3.16 ±6.212	805	36.65 ±6.212
	883	50.54 ±7.606	998	25.44 ±6.212	847	70.14 ±6.212
	829	46.81 ±6.212	840	25.02 ±6.212	816	70.01 ±6.212
	854	45.19 ±6.212	991	18.54 ±6.212	835	67.07 ±6.212
	815	43.47 ±6.212	847	17.49 ±6.212	998	62.55 ±6.212
Highest CDI	864	41.49 ±6.212	858	17.49 ±7.606	837	62.07 ±6.212
Highest CDI	887	40.14 ±6.212	868	16.10 ±6.212	850	62.07 ±6.212
	867	40.14 ±6.212	846	14.50 ±6.212	818	61.14 ±6.213
	889	40.04 ±6.213	879	13.64 ±6.213	846	60.59 ±6.212
	835	39.85 ±6.212	843	13.52 ±6.212	859	60.41 ±6.212
	830	39.70 ±7.605	825	12.90 ±6.212	883	59.11 ±6.213

Table 5: Mean and standard error of height (m) and Diameter at Breast Height (DBH) (cm) increment of the 10 families with the highest and 10 families with the lowest measurement recorded. Families from the *Eucalyptus bosistoana* families trial planted in 2012 in the Marlborough region. Increment measured between October 2020 and March 2021.

	Height i	ncrement (m)	DBH inc	rement (cm)
	Family	Mean	Family	Mean
	862	0.68 ±0.06	835	1.00 ±0.00
	849	0.65 ±0.35	813	0.63 ±0.24
	811	0.62 ±0.12	859	0.50 ±0.38
	861	0.60 ±0.25	812	0.50 ±0.20
	860	0.60 ±0.15	820	0.50 ±0.00
Largest increment	991	0.60 ±0.40	824	0.40 ±0.12
	809	0.60 ±0.15	861	0.40 ±0.17
	814	0.53 ±0.12	889	0.40 ±0.06
	810	0.50 ±0.06	804	0.40 ±0.00
	859	0.50 ±0.10	884	0.40 ±0.00
	813	0.50 ±0.15	829	0.37 ±0.18
	816	0.05 ±0.13	883	-0.30 ±0.00
	840	0.05 ±0.19	876	-0.20 ±0.00
	841	0.07 ±0.02	868	-0.15 ±0.05
	883	0.08 ±0.04	887	-0.10 ±0.20
	852	0.08 ±0.14	998	-0.05 ±0.05
	998	0.08 ±0.17	816	-0.05 ±0.05
Smallest increment	847	0.12 ±0.07	858	-0.05 ±0.15
	868	0.12 ±0.13	853	0.00 ±0.00
	835	0.13 ±0.07	877	0.00 ±0.06
	823	0.13 ±0.18	841	0.03 ±0.19
	844	0.17 ±0.07	886	0.03 ±0.09
	866	0.17 ±0.07	850	0.03 ±0.09
	873	0.18 ±0.13	854	0.03 ±0.07

Eucalyptus bosistoana 2018 clones

These data were analysed at both the family and genotype level. We observed variation in CDI and growth responses between genotypes from the same family that was not possible in the *E. bosistoana* family trial as it is of mixed, indistinguishable, genotypes. There were differences for the mean CDI score and height increment of *E. bosistoana* families (Satterthwaite's method type III ANOVA, P<0.001). Analysing differences at the genotype level showed differences in the mean CDI score and height increment of *E. bosistoana* genotypes (Satterthwaite's method type III ANOVA, P<0.001, Tables 6 and 7). Statistically, it was not possible to conduct a multiple comparison test amongst all families and genotypes, thus we used a Markov chain Monte Carlo model to test whether the best family/genotype was always significantly different from the worst family/genotype. The probability that the family with the largest mean score for a particular co-efficient is different from the smallest family was: CDI coefficient probability =0.972, height coefficient probability =1. The probability that the

genotype with the largest mean score for a particular co-efficient is different from the smallest family was: CDI coefficient probability =1, height coefficient probability =1.

There was substantial variation in the defoliation rate and growth response of different genotypes from the same family. This is consistent with our previous observation from the *E. bosistoana* 2012 family trial at Dillon where we observed substantial variation within the same family. Variation within a genotype was higher than variation within the same family (i.e., standard errors of the mean height, CDI, DBH, length and number of new shoots for genotype is wider than for the family, Table 8). Nevertheless, there was only a slight difference that could be explained by the higher number of individuals per family compared to the number of individuals per genotype. There was no correlation between CDI and height increment at an individual level (Pearson's method, cor= -0.18, P<0.001, Figure 6). A correlation test between CDI and height increment per genotype gave a few interesting results (between 4-12 samples per genotype). The genotype 11b, 11c, 122c, 15b, 17b, 19a, 55c, 58a, 817c, 819a and 8a gave strong or moderate positive or negative correlations (Table 9). A positive correlation meant that paropsine defoliation had a positive impact on the height increment. A negative correlation meant that the paropsine defoliation had a negative impact on the height increment.



Figure 6: Scatterplot of the height increment (m) and CDI score of the *E. bosistoana* clonal trial 2018 during March 2021. Each point represents one individual tree with those in the top right of the plot being most tolerant trees and those in the bottom right being most resistant to paropsine browse. The three first number of each label represent the family number. The first letter represents the genotype. The second number is the individual in each genotype. A few individuals seem to be more resistant to paropsine damage (810a, 804b, 121a, 830c1, 871a1, 822b, 830b, 836a2, 11c, 819a and 840d1). A few individuals seem to be more tolerant to paropsine damage (24a2, 17b, 55c and 868b). Label and localisation: P=plot number, T= tree number. 8a=P1T12, 10a=P1T20, 11d=P1T3, 11c=P1T10, 11b1=P3T16, 11b2=P5T11, 15c1=P2T24, 15c2=P6T21, 17b=P9T12, 24a1=P8T22, 24a2=P25T2, 29b=P25T17, 30a=P2T16, 45a=P1T8, 48a=P7T20, 53b=P24T12, 53c1=P10T7, 53c2=P24T12, 55c=P2T22, 61c1=P1T23, 61c2=P24T23, 61b=P25T23, 65a=P25T16, 121a=P1T24, 133a=P9T22, 804b=P6T25, 810a=P25T24, 814b=P1T25, 816a1=P6T12, 816a2=P12T2, 817b=P17T19, 819a=P1T21, 822b=P25T25, 825a=P7T24, 830c1=P1T19,

830a=P3T5, 830c2=P12T4, 830b=P25T4, 832a=P2T8, 839a=P24T11, 840d1=P6T2, 840d2=P25T12, 841b=P2T2, 847a=P2T20, 852a=P1T17, 852c=P4T12, 855a=P1T22, 863a1=P1T22, 863a2=P11T5, 863a3=P24T21, 866a=P2T11, 866b=P5T15, 868a=P5T9, 868b=P16T4, 868c=P24T9, 871a1=P1T13, 871a2=P25T3, 875a=P1T16, 881a=P24T4

Table 6: Mean and standard error of Crown Damage Index (CDI) score of the 12 genotypes with the lowest and 12 with the highest measurement recorded. Families from the *Eucalyptus bosistoana* clonal trial planted in 2018 in the Marlborough region. Two measurement periods: October 2020 and March 2021.

Crown Damage Index (CDI)	Genotype	October 2020	Genotype	March 2021
	17a	1.73 ±1.13	822b	38.33 ±0.96
	819a	1.95 ±1.14	10a	43.13 ±2.79
	866b	2.13 ±0.57	814b	43.50 ±6.50
	889a	2.64 ±2.25	55b	43.75 ±3.75
	45a	2.93 ±1.69	69a	44.17 ±2.50
Lowest CDI	7a	3.44 ±1.68	39b	44.44 ±5.02
Lowest CDI	22a	3.50 ±1.80	830a	45.00 ±1.36
	69a	3.56 ±0.00	839a	45.42 ±3.75
	71a	3.70 ±2.63	804b	45.83 ±5.87
	68b	3.71 ±1.75	871b	45.83 ±4.17
	889b	3.88 ±1.59	875a	46.11 ±1.47
	839a	3.90 ±1.88	121a	46.25 ±5.15
	15c	27.90 ±8.21	122b	76.25 ±3.75
	847b	27.60 ±12.33	55c	70.42 ±2.49
	13c	26.85 ±7.02	847b	70.00 ±4.41
	55c	22.17 ±5.83	15a	69.58 ±5.79
	832a	21.39 ±11.46	814c	67.08 ±2.58
	8b	20.14 ±5.08	847d	66.67 ±0.00
Highest CDI	11a	20.11 ±3.54	45b	66.25 ±4.43
	847d	18.67 ±0.00	24a	65.83 ±5.38
	11c	18.06 ±5.62	889b	65.42 ±7.77
	820a	17.32 ±8.00	820a	64.58 ±5.29
	19a	17.28 ±5.22	52a	63.89 ±2.94
	104a	17.12 ±5.14	11a	63.33 ±4.66
	7c	16.79 ±5.81	825a	63.33 ±8.90

Table 7: Mean and standard error of height increment (m) of the 13 genotypes with the highest and 13 genotypes with the lowest measurement recorded. Genotypes from the *Eucalyptus bosistoana* clonal trial planted in 2018 in the Marlborough region. Increment measured between October 2020 and March 2021

Height gain (m)	Genotype	Mean	Height gain (m)	Genotype	Mean
	863a	0.74 ±0.09		885a	0.14 ±0.06
	804b	0.49 ±0.04		122c	0.14 ±0.03
	875a	0.48 ±0.06		104b	0.15 ±0.05
	11b	0.48 ±0.08		52a	0.15 ±0.03
	839a	0.48 ±0.06		68b	0.15 ±0.05
	866b	0.48 ±0.13	Lowest increment	17a	0.18 ±0.06
Highest increment	855a	0.46 ±0.11		13a	0.19 ±0.03
	15c	0.46 ±0.10		7c	0.19 ±0.07
	24a	0.45 ±0.11		22a	0.20 ±0.04
	830c	0.45 ±0.08		69a	0.20 ±0.05
	45a	0.44 ±0.08		7a	0.20 ±0.06
	852c	0.44 ±0.10		843a	0.20 ±0.02
	11c	0.44 ±0.14		847d	0.20 ±0.00

Table 8: Mean and standard error of Crown Damage Index (CDI), height, length and number of new shoots of the 2012 *E. bosistoana* clonal trials regarding the family or the genotype. The standard error is wider for the genotype comparisons.

MEASUREMENT	Family	Genotype
	Mean	Mean
CDI	32.16 ±3.33	32.60 ±4.07
Height	1.72 ±0.13	1.73 ±0.15
Length of new shoots	20.95 ±2.37	21.07 ±2.94
Number of new shoots	5.27 ±0.60	5.28 ±0.74

Table 6: Correlation between Crown Damage Index (CDI) and height increment with significant P-values. CDI results from March 2021, Height increment from October 2020 to March 2021. No clones= number of trees measured per genotype. A positive correlation meant that the defoliation had a positive impact on the height increment. A negative correlation meant that the defoliation had a negative impact on the height increment.

Correlation CDI/height gain								
Genotype	corr.	P-value		No clones				
17b	0.9795	0.0205	*	4				
19a	0.9771	0.0229	*	4				
56a	0.9445	0.0555		4				
803a	0.9418	0.0582		4				
868b	0.9206	0.0794		4				
122c	0.6015	0.0386	*	12				
53c	-0.6752	0.0662		8				
15b	-0.7266	0.0412	*	8				
11b	-0.7504	0.0320	*	8				
58a	-0.7815	0.0220	*	8				
804b	-0.9001	0.0999		4				
810b	-0.9025	0.0975		4				
866b	-0.9173	0.0827		4				
121a	-0.9182	0.0818		4				
810a	-0.9191	0.0809		4				
8a	-0.9198	0.0012	**	8				
830c	-0.9290	0.0710	•	4				
11c	-0.9540	0.0460	*	4				
819a	-0.9679	0.0321	*	4				
817c	-0.9921	0.0321	*	4				
55c	-0.9922	0.0078	**	4				

Eucalyptus tricarpa 2017 families

There was no site or family level differences in defoliation of *E. tricarpa* (P>0.05 for both site and family, Figure 7 representing CDI measurement only). The length of new shoots, the number of new shoots and height increment did not show any site effect or differences between families (Satterthwaite's method type III ANOVA P>0.05, Figure 8 representing height increment only). A DBH increment difference between families was observed (Satterthwaite's method type III ANOVA and t-test, P<0.0014, Figure 9). Specifically, family 669 was different from families 624, 656, 662, 671, 646, 652, 657, 655, 649, 623, 654 and 658 (Multi-comparison Tukey test). There was no correlation between CDI and height increment (Pearson's method, cor=-0.27, P=0.0079), but a moderate association between CDI and DBH increment and DBH and height increment (cor=-0.44 and 0.59, P=0.0053 and 0.001 respectively, Pearson's method).

From a pest management perspective, we do not be recommend continuing with *E. tricarpa* development at this point in time. This is based on the consistently high defoliation across all families, low observed growth rate, absence of height and DBH increment differences between most families. To reconsider using this species will require investment in biological control to ensure top down control of paropsines as no tolerance and resistance strategies were observed in *E. tricarpa*.



Figure 7: Estimated mean and standard error of Crown Damage Index (CDI) score of each *E. tricarpa* family from the 2017 trial from two sites in the Marlborough region. Six trees measured per family (three in each site) Trees measured in January 2020, October 2020 and March 2021. The family 652 was the less defoliated. The family 658 was the most defoliated.



Figure 8: Estimated mean and standard error of height increment (m) of each *E. tricarpa* family from the 2017 trial from two sites in the Marlborough region. Six trees measured per family (three in each site) Trees measured in January 2020, October 2020 and March 2021. The family 649 had the smallest height increment. The family 669 had the biggest height increment.



Figure 9: Estimated mean and standard error of Diameter at Breast height (DBH) increment (cm) of each *E. tricarpa* family from the 2017 trial. Three trees measured per family. The DBH was measured only on Lissaman site (Dillon site trees too small). Trees measured in October 2020 and March 2021. The family 649 had the smallest height increment. The family 644 had the biggest height increment.

CONCLUSION

Resistant species (*E. globoidea*, *E. cladocalyx* and *E. macrorhyncha*) had the highest DBH and height increment. *E. quadrangulata* was the most defoliated species (only present at Dillon) followed by *E. camaldulensis*, *E. tricarpa* and *E. bosistoana*. The species that grew the most were *E. globoidea* for the DBH increment and *E. cladocalyx* and *E. macrorhyncha* for the height increment.

E. bosistoana presents an interesting case of tolerance due to large variation in defoliation and its relationship with growth rates between and within families and genotypes. Even though we assessed only two trials from the Marlborough region, site and plot effects were observed due to micro-climate and or paropsine amount differences. To ascertain if the variation in defoliation by paropsines and its impact on growth is heritable (families and/or genotype level) will require new plantings or assessing at larger scales. This could be done by a) planting family and genotype trials with more homogenous conditions, b) assessing more trees to have more reliable statistical results, potentially using remote sensing.

E. quadrangulata and *E. tricarpa* were highly defoliated and no difference in term of defoliation and height increment were observed between *E. tricarpa* families. Due to this absence of variation, it is unlikely that resistant/tolerant trees within these species will be found.

ACKNOWLEDGEMENTS

Fundings: Specialty Wood Products (SWP) in Partnership with New Zealand Dryland Forest Initiative (NZDFI), New Zealand Institute of Forestry (NZIF) and Entomological Society of New Zealand (ESNZ).

Collaborators: Paul Millen (NZDFI), Ruth Mcconnochie (NZDFI), Shaef Van Ballekom (Proseed), Paul Shroeder (Proseed), Elena Moltchanova (UC), Luis Apiolaza (UC), Ning Ye (UC), Carolin Weser, Vega Xu (UC), Josh McCulloch (UC), Kelvin Barsdale (UC), Marie Squire (UC), Amanda Inglis (UC), Meike Holzenkämpfer (UC), David Conder (UC), Brett Robinson (UC), Robert Stainthorp (UC), Gert Hendriks (UC), Monika Sharma (UC), Geoffrey Gaudou.

Supervision: Stephen Pawson, Justin Morgenroth.

APPENDIX

Species trial

Table A1: Mean and standard error of the CDI score, DBH and height increment (from December 2019 to March 2021 gain change), length and number of new shoots (total length values from tip to October growth ring) of 7 Eucalyptus species in two sites of the Marlborough region, NZ. Absolute values of three measurement periods (December 2019, October 2020 and March 2021). Mean CDI= mean of the Crown Damage Index (defoliation level). Mean DBH gain= mean of the Diameter at Breast Height (1.40m). Mean length shoots = mean of the longest shoots from the three cut shoots, mean number shoots = mean of the number of stems counted for the three cut shoots. E. macrorbyncha, E. cladocalyx and E. globoidea were the least defoliated with the shortest new shoots length and had the higher height and DBH. E. quadrangulata, E. camaldulensis, E. tricarpa and E. bosistoana were the most defoliated, with the longest new shoots and with the smallest height and DBH. The number of shoots did not show any variation between species. There is some NA datas for December 2019. Increment measure was not possible during the first assessment (need at least two measurments).

Species	Date	Site	Mean CDI	Mean height gain	Mean DBH gain	Mean length shoots	Mean number shoots
E. bosistoana							
	Dec.	Dillon	47.89± 4.58	NA	NA	NA	NA
	2019	Lissaman	43.11± 1.97	NA	NA	NA	NA
	Oct.	Dillon	10.62± 2.44	0.15± 0.04	0.45±0.09	13.81 ±1.37	9.91 ±1.47
	2020	Lissaman	3.38± 0.52	0.34± 0.04	0.38± 0.05	26.31 ±1.88	10.18 ±0.77
	Mar.	Dillon	49.89± 3.24	0.18± 0.03	0.42 ± 0.07	16.49 ±1.34	10.13 ±0.79
	2021	Lissaman	34.78± 1.63	0.41± 0.07	0.13 ± 0.04	33.42 ±2.06	6.47 ±0.43
Mean CDI Dillon		Dillon	36.71 ±3.39	0.17 ±0.02	0.44 ±0.06	13.81 ±1.37	10.02 ±0.82
Mean CDI Lissaman		Lissaman	27.09 ±2.71	0.38 ±0.04	0.25 ±0.04	26.31 ±1.88	8.32 ±0.55
Overall mean			31.85 ±2.21	0.27 ±0.03	0.34 ±0.04	20.06 ±1.63	9.17 ±0.50
E. camaldulensis							
	Dec.	Dillon	46.63± 4.92	NA	NA	NA	NA
	2019	Lissaman	48.96± 3.30	NA	NA	NA	NA
	Oct.	Dillon	21.50± 4.40	0.38± 0.12	0.72±0.36	8.32 ±0.92	11.44 ±1.49
	2020	Lissaman	11.04± 1.99	0.23± 0.04	0.14 ± 0.03	22.96 ±1.68	10.77 ±1.10
	Mar.	Dillon	53.88± 3.92	0.57± 0.21	0.06 ± 0.03	18.53 ±1.31	11.67 ±0.93
	2021	Lissaman	35.52± 1.69	0.35± 0.06	0.65± 0.18	34.67 ±2.32	7.65 ±1.17
Mean CDI Dillon		Dillon	40.67 ±3.26	0.49 ±0.14	0.39 ±0.19	8.32 ±0.92	11.56 ±0.86
Mean CDI Lissaman		Lissaman	31.84 ±2.67	0.29 ±0.04	0.41 ±0.11	22.96 ±1.68	9.21 ±0.84
Overall mean			36.11 ±2.13	0.37 ±0.06	0.40 ±0.11	15.88 ±1.64	10.34 ±0.61
E. cladocalyx							
	Dec.	Dillon	23.70± 2.89	NA	NA	NA	NA
	2019	Lissaman	7.98± 1.32	NA	NA	NA	NA
	Oct.	Dillon	1.45± 0.53	0.57± 0.20	0.97±0.09	9.07 ±1.69	5.07 ±0.96
	2020	Lissaman	0.11± 0.04	0.50± 0.07	0.74± 0.09	12.13 ±1.58	6.96 ±0.48
	Mar.	Dillon	29.52± 3.01	0.96± 0.13	0.57± 0.07	31.27 ±1.19	9.04 ±0.85
	2021	Lissaman	9.98± 2.05	0.27± 0.04	0.39± 0.04	40.42 ±2.66	8.93 ±0.80

26

SWP-T140 Eucalyptus resistance to paropsine beetles_G3.docx

Species	Date	Site	Mean CDI	Mean height gain	Mean DBH gain	Mean length shoots	Mean number shoots
Mean CDI Dillon		Dillon	18.22 ±2.28	0.78 ±0.12	0.77 ±0.07	9.07 ±1.69	7.06 ±0.73
Mean CDI Lissaman		Lissaman	6.16 ±1.03	0.39 ±0.05	0.56 ±0.06	12.13 ±1.58	7.94 ±0.50
Overall mean			12.26 ±1.41	0.58 ±0.07	0.67 ±0.05	10.60 ±1.17	7.50 ±0.44
E. globoidea							
	Dec.	Dillon	17.98± 3.70	NA	NA	NA	NA
	2019	Lissaman	38.80± 3.89	NA	NA	NA	NA
	Oct.	Dillon	6.34± 1.38	0.28±0.07	1.31±0.12	5.08 ±0.89	6.51 ±1.67
	2020	Lissaman	2.78±0.75	0.38±0.12	0.71±0.09	18.38 ±1.29	9.22 ±0.68
	Mar.	Dillon	34.50±2.87	0.85±0.11	0.90± 0.12	27.40 ±1.92	9.56 ±0.74
	2021	Lissaman	28.83±1.29	0.50±0.09	0.33±0.07	29.87 ±1.24	5.78 ±0.42
Mean CDI Dillon		Dillon	19.61 ±2.36	0.60 ±0.09	1.10 ±0.09	5.08 ±0.89	8.03 ±0.94
Mean CDI Lissaman		Lissaman	23.47 ±2.66	0.44 ±0.07	0.52 ±0.07	18.38 ±1.29	7.50 ±0.51
Overall mean			21.54 ±1.78	0.52 ±0.06	0.81 ±0.07	11.73 ±1.46	7.77 ±0.53
E.							
macrorhyncha	D	Dillon	10 04 1 0 40		N1.0		
	Dec. 2019		16.84± 6.48				NA
		Lissaman Dillon	6.44± 2.11 4.29± 1.87	NA 0.27± 0.08	NA 0.97± 0.14	NA 7.42 ±1.13	NA 7.24 ±1.23
	Oct. 2020	Lissaman	4.29 ± 1.87 0.25 ± 0.14	0.27 ± 0.08 0.41 ± 0.06	0.97 ± 0.14 0.83 ± 0.07	7.42 ±1.13 11.04 ±0.81	7.24 ±1.23 8.93 ±1.02
	Mar.	Dillon	0.23± 0.14 20.91± 4.55	0.41 ± 0.00 0.86 ± 0.10	0.83 ± 0.07 0.63 ± 0.13	31.20 ± 2.30	7.47 ±0.79
	2021	Lissaman	9.16± 1.97	0.57± 0.10	0.03 ± 0.13 0.30 ± 0.04	40.51 ±2.19	6.00 ±0.47
Mean CDI Dillon	2021	Dillon	14.71 ±3.01	0.59 ±0.09	0.80 ±0.10	7.42 ±1.13	7.36 ±0.72
Mean CDI		Lissaman	5.28 ±1.10	0.49 ±0.04	0.56 ±0.06	11.04 ±0.81	7.47 ±0.62
Lissaman Overall mean			9.83 ±1.63	0.53 ±0.05	0.68 ±0.06	9.23 ±0.76	7.41 ±0.47
<i>E.</i>			5.05 ±1.05	0.00 ±0.00	0.00 ±0.00	5.25 ±0.70	/.41 ±0.4/
quadrangulata							
	Dec. 2019	Dillon	50.67± 3.88	NA	NA	NA	NA
	Oct. 2020	Dillon	27.67± 2.58	0.25±0.04	0.37±0.09	18.18 ±1.07	10.33 ±1.55
	Mar.	Dillon	66.67± 1.88	0.22± 0.03	0.39± 0.11	21.38 ±1.89	11.84 ±1.28
Mean Dillon	2021	Dillon	48.34 ±2.91	0.23 ±0.03	0.38 ±0.07	18.18 ±1.07	11.09 ±1.00
E. tricarpa							
	Dec.	Dillon	56.11± 2.73	NA	NA	NA	NA
	2019	Lissaman	49.18± 2.95	NA	NA	NA	NA
				0.18± 0.04	0.65± 0.15	11.93 ±1.32	13.07 ±1.53
	Oct	Dillon	4.64± 0.77	0.18± 0.04	0.00 0.10		
	Oct. 2020	Dillon Lissaman	4.64± 0.77 1.85± 0.25				
	2020	Lissaman	1.85± 0.25	0.32±0.03	0.53± 0.09	23.08 ±1.11	10.48 ±1.04

27

Species	Date	Site	Mean CDI	Mean height gain	Mean DBH gain	Mean length shoots	Mean number shoots
Mean CDI Lissaman		Lissaman	30.25 ±3.27	0.37 ±0.04	0.37 ±0.06	23.08 ±1.11	8.88 ±0.65
Overall mean			34.68 ±2.57	0.33 ±0.03	0.40 ±0.05	17.69 ±1.32	10.76 ±0.59

Eucalyptus bosistoana 2012 family trial



Figure A1: Estimated mean and standard error of CDI score of each *E. bosistoana* family from the 2012 trial. Three trees per family measured in December 2019, October 2020 and March 2021. The family 991 was the less defoliated. The family 883 was the most defoliated.



Figure A2: Estimated mean and standard error for the height increment (m) of each *E. bosistoana* family from the 2012 trial. Three trees per family measured in October 2020 and March 2021. The family 816 had the smallest height increment. The family 826 had the biggest height increment.



Figure A3: Estimated mean and standard error for the DBH increment (cm) of each E. bosistoana family from the 2012 trial. Three trees per family measured in October 2020 and March 2021. The family 876 had the smallest DBH increment. The family 835 had the biggest DBH increment.

30

Table A2: Mean and standard error of CDI score of the 10 families with the lowest and 10 with the highest measurement recorded. Families from the *Eucalyptus bosistoana* family trial planted in 2012 in the Marlborough region. Three measurement periods: December 2019, October 2020 and March 2021.

Measurement						
Defoliation level (CDI)	Family	December 2019	Family	October 2020	Family	March 2021
	991	14.04 ±6.213	830	1.28 ±6.213	996	32.45 ±6.213
	869	15.84 ±6.212	890	1.34 ±6.212	854	33.19 ±6.212
	859	18.19 ±6.212	804	1.63 ±6.214	884	33.88 ±6.218
	846	18.71 ±6.213	859	2.26 ±6.212	819	34.49 ±6.212
Lowest CDI	839	18.99 ±6.212	884	2.35 ±6.218	802	35.14 ±6.212
LOWEST CDI	805	19.69 ±6.212	865	2.58 ±6.212	873	35.73 ±5.381
	842	19.98 ±7.606	833	2.84 ±6.212	862	35.91 ±6.212
	855	20.04 ±6.213	801	2.95 ±6.212	801	36.08 ±6.212
	809	20.17 ±6.212	886	3.09 ±6.212	830	36.51 ±6.213
	826	21.33 ±6.212	805	3.16 ±6.212	805	36.65 ±6.212
	883	50.54 ±7.606	998	25.44 ±6.212	847	70.14 ±6.212
	829	46.81 ±6.212	840	25.02 ±6.212	816	70.01 ±6.212
	854	45.19 ±6.212	991	18.54 ±6.212	835	67.07 ±6.212
	815	43.47 ±6.212	847	17.49 ±6.212	998	62.55 ±6.212
Highest CDI	864	41.49 ±6.212	858	17.49 ±7.606	837	62.07 ±6.212
nighest CDI	887	40.14 ±6.212	868	16.10 ±6.212	850	62.07 ±6.212
	867	40.14 ±6.212	846	14.50 ±6.212	818	61.14 ±6.213
	889	40.04 ±6.213	879	13.64 ±6.213	846	60.59 ±6.212
	835	39.85 ±6.212	843	13.52 ±6.212	859	60.41 ±6.212
	830	39.70 ±7.605	825	12.90 ±6.212	883	59.11 ±6.213

Eucalyptus bosistoana 2018 clonal trial



Figure A4: Estimated mean and standard error of CDI score of each *E. bosistoana* family from the 2018 clonal trial. Trees measured in October 2020 and March 2021. The family 828 was the less defoliated. The family 832 was the most defoliated.



Figure A5: Estimated mean and standard error for the height increment (m) of each *E. bosistoana* family from the 2018 clonal trial. Trees measured in October 2020 and March 2021. The family 885 had the smallest height increment. The family 863 had the biggest height increment.



Figure A6: Estimated mean and standard error of CDI score of each *E. bosistoana* genotypes from the 2018 clonal trial. Trees measured in October 2020 and March 2021. The genotype 68b was the less defoliated. The genotype 15c was the most defoliated.

SWP-T140 Eucalyptus resistance to paropsine beetles_G3.docx



Figure A7: Estimated mean and standard error of height increment (m) of each *E. bosistoana* genotypes from the 2018 clonal trial. Trees measured in October had the biggest height increment.

Genotype

REFERENCES

- De Jong, T. J., & Van Der Meijden, E. (2000). On the correlation between allocation to defence and regrowth in plants. *Oikos, 88*(3), 503-508.
- dos Santos Bobadilha, G., Vidaurre, G. B., Câmara, A. P., Neto, H. F., da Silva Oliveira, J. T., Soliman, E. P., . . . Zanuncio, J. C. (2019). Effect of defoliator insect on growth and wood properties of eucalypt trees. *European Journal of Wood and Wood Products*, *77*(5), 861-868.
- Iqbal, N., Masood, A., & Khan, N. (2012). Analyzing the significance of defoliation in growth, photosynthetic compensation and source-sink relations. *Photosynthetica*, *50*(2), 161-170.
- Jolivet, P. H., Cox, M. L., & Petitpierre, E. (1994). *Novel aspects of the biology of Chrysomelidae* (Vol. 50): Springer Science & Business Media.
- Karen J. Marsh, I. S., Charles H. Hocarta, Kara Youngentob, Inder-Pal Singh, William J. Foley. (2019). Occurrence and distribution of unsubstituted B-ring flavanones in *Eucalyptus* foliage. *Phytochemistry*, 160, 31-39.
- Leimu, R., & Koricheva, J. (2006). A meta-analysis of tradeoffs between plant tolerance and resistance to herbivores: combining the evidence from ecological and agricultural studies. *Oikos, 112*(1), 1-9.
- Lin, H. (2017). *Risk and Impact of Insect Herbivory on the Development of Dryland Eucalyptus Forestryin New Zealand*, Canterbury University).
- O'Reilly-Wapstra, J., McArthur, C., & Potts, B. (2004). Linking plant genotype, plant defensive chemistry and mammal browsing in a *Eucalyptus* species. *Functional Ecology, 18*(5), 677-684.
- Reich, P. B., & Borchert, R. (1984). Water stress and tree phenology in a tropical dry forest in the lowlands of Costa Rica. *The Journal of Ecology*, 61-74.
- Restif, O., & Koella, J. C. (2004). Concurrent evolution of resistance and tolerance to pathogens. *The American Naturalist, 164*(4), E90-E102.
- Simon P. Whittock, L. A. A., C. M. Kelly, B. M. Potts. (2003). Genetic control of coppice and lignotubers development in *Eucalyptus globulus*. *Australian Journal of Botany, 51*, 57-67.
- Stone, C. (2001). Reducing the impact of insect herbivory in eucalypt plantations through management of extrinsic influences on tree vigour. *Austral Ecology*, *26*(5), 482-488.
- Stone, C., Matsuki, M., Carnegie, A. J., Stone, C., Matsuki, M., & Carnegie, A. (2003). *Pest* and disease assessment in young eucalypt plantations: field manual for using the *Crown Damage Index*: Bureau of Rural Sciences.
- White, T. t. (1984). The abundance of invertebrate herbivores in relation to the availability of nitrogen in stressed food plants. *Oecologia, 63*(1), 90-105.
- Withers, T. (2001). Colonization of eucalypts in New Zealand by Australian insects. *Austral Ecology*, *26*(5), 467-476.
- Withers, T., & Peters, E. (2017). 100 years of the eucalyptus tortoise beetle in New Zealand. *New Zealand Journal of Forestry, 62*, 16-20.