



Technical Report

Eucalyptus resistance to paropsine beetles.

Author/s: Leslie Mann and Stephen Pawson

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TABLE OF CONTENTS

EXECUTIVE SUMMARY	1
INTRODUCTION	2
Paropsine beetles (Paropsis charybdis (Stål) and Paropsisterna cloelia (Stål))	2
Resistance or tolerance to defoliation	2
METHODS	4
Tree grading	4
Pest Čounting	4
Crown Damage Index (CDI)	4
Diameter at breast height	5
Tree height	5
RESULTS and Discussion	6
Trial 1: Species resistance to paropsine beetles	6
Trials 2 and 3: <i>E. bosistoana</i> family resistance to paropsine beetles	10
Trial 2: 2012 E. bosistoana	10
Trial 3: <i>E. bosistoana</i> clones trial	12
Trial 4: <i>E. tricarpa</i> family resistance to paropsine beetles	15
CONCLUSION	18
ACKNOWLEDGEMENTS	19
REFERENCES	19

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EXECUTIVE SUMMARY

A wide range of insects can infest *Eucalyptus* trees in New Zealand, particularly pest insects that are native to Australia. Australian paropsine beetles are significant pests in New Zealand where climatic conditions are similar to their native range and they have few natural enemies. To date, five paropsine species have successfully established in New Zealand. *Paropsis charybdis* and *Paropsisterna cloelia* (EVB) are most frequently observed in *Eucalyptus* plantations and cause the most damage. In 2019, EVB was still restricted to the South-East of the North Island, however it spread to the upper South Island in the summer of 2020. Both species cause considerable damage within plantations. *Eucalyptus* tolerance to insect defoliation is poorly understood, but must be quantified across species and families to select appropriate breeding lines for the New Zealand Dryland Forest Initiative (NZDFI) to establish a healthy, productive and durable *Eucalyptus* timber industry in New Zealand.

We aim to determine if specific *Eucalyptus* species or families (genetic lines) are more resistant or tolerant to paropsine browse. To answer this we have made year 1 assessments of paropsine defoliation among seven different *Eucalyptus* species (*E. quadrangulata, E. bosistoana, E tricarpa, E. globoidea, E. macrorhyncha, E. camaldulensis* and *E. cladocalyx*), clones of *E. bosistoana*, and families of *E. tricarpa and E. bosistoana*.

Key results:

- The Crown Damage Index (CDI) is the best technique (tradeoff between precision and efficiency) currently available to assess paropsine defoliation of *Eucalyptus*.
- CDI results show clear differences in the susceptibility of *Eucalyptus* species to defoliation at Dillon and Lissaman sites. *E. tricarpa, E. quadrangulata, E. camaldulensis,* and *E. bosistoana* were heavily defoliated, *E. macrorhyncha,* and *E. cladocalyx* had low levels of defoliation and *E. globoidea* was intermediate between these two groups.
- There was substantial range in the defoliation within a species and individual outliers with minimal defoliation are candidates for further investigation.
- Baseline DBH and height measurements were taken to inform future estimates of tolerance after repeated sampling.
- Within family variability to defoliation of *E. bosistoana* was high, in part due to the low number of replicates at the Dillon trial. The rank order of defoliation amongst families provides a guide for further investigation of optimal families for breeding decisions.
- Variability in defoliation amongst *E. bosistoana* clones was less than the intra-family variability. The rank order of defoliation amongst clones identifies those clones that are most resistant to paropsine browse.
- Assessment of *E. tricarpa* families showed consistently high levels of defoliation that was consistent with that observed in the multiple species trial.

Work to date has provided estimates of the resistance of individual species, families, provenances or clones to paropsine browse. We now have baseline measures of height and DBH that can be remeasured in the spring/summer of 2020 to 2021. This concurrent remeasurement of both defoliation and growth will allow us to further refine our understanding of resistance and present the first result of paropsine tolerance.

INTRODUCTION

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

The tribe Paropsini (paropsine leaf beetles) has over 400 species, most native to Australia where they feed almost exclusively on eucalypts (Jolivet, Cox, & Petitpierre, 1994). Five eucalypt feeding paropsine species (*Paropsis charybdis, Trachymela sloanei* (Blackburn), *Trachymela catenata* (Chapuis), *Paropsisterna cloelia* and *Paropsisterna beata* (Newman)) have established in New Zealand, however the status of *P. beata* is uncertain and may no longer be present. *Paropsis charybdis* and *Pst. cloelia* are the most significant pest species. Although the eggs, larvae and adult of each species are easily segregated, their feeding damage cannot be distinguished. *Paropsis 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 was observed in the South Island in 2019 (New Zealand Farm Forestry Association, 2019) . Lin et al. (2017) found that the newly established *Pst. cloelia* was more abundant than *P. charybdis* and caused greater defoliation. This was tentatively attributed to the longer activity period of *P. cloelia* compared to *P. charybdis*.

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). Weather conditions, soil composition, nitrogen leaf availability and leaf toughness are also crucial for paropsine beetles. *Eucalyptus* growing in non-fertile soil 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 to recover, 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 to insects. For example, stress, such as drought can induce a compensatory response that increases nitrogen and carbohydrate content that ultimately benefits herbivores (White, 1984).

Resistance or tolerance to defoliation

The NZDFI breeding programme aims to develop trees with improved growth rate and wood properties, particularly durability. Resistance or tolerance to paropsine browse is critical to the improvement of tree 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 defoliation and recover from defoliation events with little loss of growth. Resistance differences could be explained by variation in the chemical compounds present in the leaves. If a tree is highly toxic, few insect species will feed on it as they have to develop specialized strategies to overcome the plant toxins. Tolerance can be explained by a higher nitrogen and carbohydrates stock, and potentially a higher photosynthetic rate, which cumulatively provides opportunities to grow new leaves in response to defoliation. Specialized insects can adapt themselves to resistant tree species or families. Conversely tree species or tree families can adapt to herbivory as well. Thus an evolutionary "arms race" can develop with perpetual selection pressures acting on attributes of either the insect herbivore or the affected tree. Attributes of tree tolerance do not include adaptation by the herbivore, thus leading to a more stable coevolution that can be incorporated into a breeding programme.

Lin (2017) began assessing resistance of different *Eucalyptus* species and *E. bosistoana* families. Lin (2017) showed that *E. cladocalyx*, *E. macrorhyncha* and *E. globoidea* had the least paropsine browse amongst eleven species assessed (Figure 1). Lin (2017) also assessed the tolerance of 14 *E. bosistoana* families to artificial defoliation of the tree crown. Lin (2017) observed large variation in the growth response following artificial defoliation and the impact on tree growth was larger when defoliation occurred in late spring and /or several times during the year. However, Lin (2017) did not measure tolerance amongst other *Eucalyptus* species within the NZDFI breeding programme and did not measure the effect of insect derived defoliation.

Here we present the first results of the PhD work of Leslie Mann. Specifically we present the results of the first season of *Paropsis* browse on a range of *Eucalyptus* species and families. Future work will measure the impact of this defoliation (and on-going defoliation) on growth over time. An assessment of tolerance will not be available until growth measurements in early summer 2020 are completed. Assessing growth at the start of the 2020 summer will quantify the impact of 2019 herbivory on growth.



Figure 1: Proportion of inspected trees assigned to each level of chewing damage (a = no or little chewing (<5%); b = light chewing (5-25% defoliation); c = moderate chewing (26-50% defoliation); d = moderately severe chewing (51-60% defoliation) for each Eucalyptus species across three study sites. The number of trees assessed at each site is indicated by n beside each species name respectively (H Lin, Murray, & Mason, 2017).

METHODS

We assess insect presence and defoliation amongst seven *Eucalyptus* species (*E. quadrangulata*, *E. bosistoana*, *E tricarpa*, *E. globoidea*, *E. macrorhyncha*, *E. camaldulensis* and *E. cladocalyx*), 86 *E. bosistoana* families, and 16 *E. tricarpa* families using three methods, tree grading, pest counting and the crown damage index (CDI). These three measures of insect abundance and damage allow us to assess resistance. We trial the three methods in the first summer season to assess the most practical method to implement in subsequent years. In addition we measure baseline information of tree size, i.e., diameter at breast height and tree height, to provide a reference point against which tolerance to paropsine browse can be assessed. Fieldwork will occur during the summer season when paropsine beetles are active. Different methods have been used to quantify herbivory within *Eucalyptus*. Most commonly visual ground assessment are applied (Stone et al., 2003). However, other methods, such as chemical analyses and growth rates, have been used (dos Santos Bobadilha et al., 2019; Karen J. Marsha, 2019; O'Reilly-Wapstra, McArthur, & Potts, 2004).

Field-based resistance/tolerance experiments are divided into four trials:

Trial 1: The Dillon (planted 2012) and Lissaman (planted 2013) trial sites were assessed between 11th and 18th January 2020 for differences amongst *Eucalyptus* species. Thirty trees were assessed per species (15 trees per site).

Trial 2: 219 trees (three trees per family) from 73 *E. bosistoana* families that represented 6 provenances were assessed at the 2012 planting at Dillon.

Trial 3: 620 clones from 73 *E. bosistoana* families that represented 15 provenances at the NZDFI Dillon (planted 2018) clonal trial site were assessed. Clones are genetically identical, hence are likely to be less variable in their response to defoliation. Thus, clones from the same individual should not respond differently to herbivory.

Trial 4: Sixteen *E. tricarpa* families (three trees per family per site) were assessed at the 2017 plantings from the Dillon and Lissaman trial sites.

Tree grading

This is the simplest and fastest visual assessment of chewing damage. Because the most damaging insects (*P. charybdis* and *Pst. cloelia*) are chewing insects then we can use this method to estimate canopy loss to herbivory. Tree grading is subjective and thus requires specific training to ensure assessment is consistent between observers. Tree grading is fast and allows researchers to assess many trees in a short time frame. Tree grading is a coarse measure that assigns four different levels of upper crown defoliation: a) little or no defoliation, b) light defoliation, c) moderate defoliation and d) severe defoliation.

Pest Counting

The pest counting method is a visual defoliation estimation of three shoots per tree where number of eggs, larvae stage (early, mid, late instar and pupae) and adult are recorded. The different paropsine species (*P. charybdis*, *Pst. cloelia* and *T. sloanei*) are recorded. Lin (2017) found that the pest counting per shoot was the most accurate. Although quantitative, and by extension, statistically better, pest counting is time consuming and difficult to implement for large trees.

Crown Damage Index (CDI)

The CDI method is a visual defoliation estimate of the entire tree. Developed and validated in Australia, it is the most common method to assess *Eucalyptus* defoliation (Stone et al., 2003). The CDI is only possible when the crown is visible and estimation can be subjective and potentially prone to observer bias. The CDI shoot assessment is a derivative that evaluates only 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 shoots. Because we assess only paropsine beetle damage, only shoots from the upper crown where beetles are active is assessed. The CDI score is called the health score that is calculated as the (Incidence*Severity)/100. The incidence is the mean observed percent damage to leaves of the three shoots. The severity is the average damage per leaf of all three shoots (Stone et al., 2003). Higher CDI scores indicate more severe defoliation.

Diameter at breast height

DBH will be measured at 1.4 m from the ground with a calibrated diameter tape or a tree calliper, depending on the tree size. This measure has to be taken during the first summer season at the same time as the resistance assessment to permit future estimates of tolerance.

Tree height

Tree height will be measured with a vertex or a staff depending on the tree size. Like DBH this must be measured in the first summer to allow future comparisons as an indicator of tolerance.

RESULTS AND DISCUSSION

Trial 1: Species resistance to paropsine beetles

Pest counting: Only adult individuals were present during the sampling period. Adult abundance differed between the sites with a mean of 3.97 at Dillon and 0.95 and Lissaman (ANOVA, F= 10.12, P<0.01). There was a significant difference in adult number for *E. tricarpa* only (Linear mixed model, d.f=19.67, P<0.05, Figure 2).



Figure 2: Mean number of adult paropsine present on three shoots per species. Pest counting method, 30 trees with two sites per species assessed. Linear mixed model analysis. Results show a difference in term of adult number between the two sites (P<0.01).

Tree grading: Assessing herbivory using tree grading identified three different groups:

- 1. Highly defoliated (*E. bosistoana, E. camaldulensis and E. tricarpa*) with more than 30 trees with c-d grading.
- 2. Moderate damage in *E. cladocalyx, E. globoidea* and *E. quadrangulata,* with 15-21 trees in the c-d grading.
- 3. Least defoliation amongst *E. macrorhyncha*, with only 6 trees in the c-d grading.

		Gro	pup 1	Group 2			Group 3
Tree grad.	E. bosis.	E. cama.	E. tric.	E. clado.	E. glob.	E. quadr.	E. macr.
а	0	0	0	1	1	0	7
b	0	0	0	13	8	0	17
С	5	3	10	15	10	4	5
d	25	28	21	1	11	11	1

_Table 1: Total of the different tree grading levels regarding the species

CDI: Site (Dillon and Lissaman) did not influence the CDI scores (ANOVA, F=0.0881, P=0.77). CDI score was different between species (ANOVA, F=7.7482, P<0.001) with two broad groups. Firstly *E. macrorhyncha, E. cladocalyx* and *E. globoidea* had comparatively low levels of defoliation and secondly, *E. bosistoana, E. camaldulensis, E. quadrangulata* and *E. tricarpa* that had higher levels of defoliation (Figure 3). Defoliation was least in *E. macrorhyncha, E. globoidea* and *E. cladocalyx* and this was distinct from the other four species assessed (Linear mixed model, P<0.05). This first group (in particular *E. macrorhyncha* and *E. cladocalyx*) can be considered to be the most resistant to paropsine defoliation of the NZDFI species that we tested.



Figure 3: Defoliation level as a function of species using the Crown Damage Index. Higher values indicate greater defoliation.

Although the *E. bosistoana, E. camaldulensis, E. quadrangulata* and *E. tricarpa* were the least resistant to paropsine browse we do not yet know the relative tolerance of these species to browse and its impact on growth. Our results are consistent with Lin (2017) who examined resistance to paropsine browse amongst various *Eucalyptus* species in the North Island. Repeatability between the North Island and South Island is promising in that it suggests that these differences are genetically controlled to some degree. Of particular interest are the outliers at the bottom of the box and whisker plots (Figure 3) these represent individual trees that may be promising for further investigation as they had much less defoliation than others of that species. This may be a random effect, but once their tolerance has been assessed in the 2020 summer then we can examine the performance of these individuals.

Height and DBH: As expected there were site differences in growth that largely reflected the older planting at Dillon (P<0.001). However, this was not consistent across all species with younger *E. bosistoana* trees at Lissaman being taller than the older plantings of *E. bosistoana* at Dillon (Figure 4). Comparing height between species at Dillon, *E. bosistoana* was shorter than *E. camaldulensis*, *E. cladocalyx*, *E. globoidea* and *E. macrorhyncha* (Multiple comparison "Tukey, P>0.05). However, at Lissaman, no difference between species was observed (x=multiple comparison, all P>0.14).

The only species level difference in DBH within a site was *E. bosistoana* that was smaller than *E. cladocalyx*, *E. globoidea* and *E. macrorhyncha* species at Dillon (Multiple comparison "Tukey", P<0.05). No DBH differences occurred between species at Lissaman. The three species considered

as resistant (*E. macrorhyncha*, *E. cladocalyx* and *E. globoidea* (Figure 3) were the taller (Figure 4) and had larger DBH (Figure 5) than those that had a higher CDI score, which reflected heavier paropsine browse.



Figure 4: Height as a function of species. 30 trees with two sites per species assessed. ANOVA and Multiple comparison "Tukey. Results show a difference in term of height between sites (P<0.001), and in Dillon site between species as well (P>0.05).



site 軴 Dillon 軴 Lissaman

Figure 5: DBH calculated from 30 trees per species per site. ANOVA and multiple comparison "Tukey" show that only *E. bosistoana* differs from all other species but only at the Dillon site only (P<0.05).

Trials 2 and 3: *E. bosistoana* family resistance to paropsine beetles.

Trial 2: 2012 E. bosistoana

The *E. bosistoana* family trial was assessed between 14th and 15th December 2019. In total 73 families of the 2012 Dillon planting were assessed with 1 to 3 trees measured per family. Pest counting was only possible for adults as no larvae or eggs were present during the assessment period. There number of adult paropsines differed between *E. bosistoana* families (ANOVA, F=1.4989, P<0.05) (Figure 6). Families 208, 824, 843, 859, 870 and 873 had a greater number of adult individuals than the other families. Families 873 and 870 were the most infested (mean of 5 and 3.66 insects respectively), however many families had no infestation at all (see Figure 3). Mean CDI scores as a measure of defoliation showed differences between families (ANOVA, F=16.425, P<0.001). However, within family variability was high, in part due to the low number of replicates at the Dillon trial (Figure 7). The rank order of defoliation as expressed by the CDI provides an indication of resistance to paropsine browse whereby families with lower CDI scores should be considered amongst breeding decisions once tolerance has been assessed (Figure 7). The defoliation level (ANOVA, F=167.54, P<0.001) and the number of adults (ANOVA, F=5.7286, P<0.001) between families were different. Surprisingly, provenances with the highest defoliation did not have the most number of adult individuals (Figure 8).



Figure 6: Number of adult paropsine as a function of family. Pest counting method, with two sites and 73 families assessed. Linear mixed model and ANOVA analysis. Results show differences in term of adult number between families (P<0.05).



Figure 7: Defoliation level as a function of family. Crown damage Insect method in one site with 73 families assessed. Linear mixed model And ANOVA analysis. Results show differences in term of defoliation level between families (P<0.001).



Figure 8: Defoliation level as measured by the CDI for different *E. bosistoana* provenances (left panel). Mean number of adult individuals per three shoots for different *E. bosistoana* provenances (Right Panel).

Trial 3: E. bosistoana clones trial

The *E. bosistoana* clonal trial at Dillon was assessed from the 9th to 13th December 2019. Clones from 73 *E. bosistoana* families that were planted in 2018 were assessed.

Pest counting of eggs (ANOVA, F=2.4482, P<0.001), larvae (ANOVA, F=129.91, P<0.001), and adults (ANOVA, F=2.2317, P<0.001) showed large differences between families and clones, but within them as well. Some families had almost no insects whereas others had more than 50 eggs or larvae present on just three shoots. Within family differences in the number of insects present was larger than those within a given clone. This is promising in that it provides further support for a degree of genetic inheritance as individuals of a given clone were behaving more similar than the family level that represented multiple clones.

Few families were consistent in the number of eggs or larvae (Figure 9 & 10), one example of consistency with high pest loading was family 825. However, other families, like 46, had very different numbers of eggs and larvae that may reflect the sensitivity of the pest counting method to the time of sampling. Similar inconsistency was observed amongst provenances (Figure 11). Assessing paropsine resistance by sampling first instar larvae is optimal as that ensures the effect of egg parasitism on the potential future population size is incorporated. However, standardising sampling on the base of pest counting is inherently difficult as it would not rely on a Gregorian calendar but reflect biological timing that is dependent on climate, i.e., warmer temperatures mean insects develop faster. Thus the optimal sampling time will differ in any given year. Work during this trial has shown that although pest counting is more quantitative, pest counting is too time consuming and dependent on seasonality to be a reliable method for assessing tree resistance to paropsine browse. We will no longer use the pest counting method in future studies. Clones from each family were not assessed for CDI as trees were not defoliated at the time of sampling and we plan to make a CDI, height and DBH assessment in the 2020-21 summer of this trial.



Figure 9: Eggs as a function of family. Crown damage Insect method in one site with 73 families assessed. Linear mixed model analysis. Results differences in term of eggs number between families (P<0.001).



Figure 10: Number of larvae paropsine as a function of family. Pest counting method, in one site with 73 families assessed. Linear mixed model and ANOVA analysis. Results show difference in term of larvae between families (P<0.001).



Figure 11: Number of paropsine larvae and eggs as a function of provenance with 73 families assessed across the 15 provenances. Linear mixed model and ANOVA analysis. Results show difference in term of larvae and eggs between provenances (both P<0.001). Graph classified regarding the higher mean number of larvae individuals per provenance.

Trial 4: *E. tricarpa* family resistance to paropsine beetles

The *E. tricarpa* trial at the 2017 Dillon and Lissaman plantings were assessed between 13th and 15th January 2020. In total 16 families were assessed with 1 to 8 trees available for sampling per family.

The CDI as a measure of defoliation did not differ between families within or between sites (Figure 12). Defoliation was severe which was consistent with the high CDI scores observed when sampling the species trial (Figure 3) and those by Lin (2017) in North Island (Figure 1). *Eucalyptus tricarpa* has low resistance to parsopsine beetles and is highly defoliated irrespective of family at the Marlborough sites assessed. There was no difference in tree height between families but there was a strong site effect with trees at Lissaman being substantially taller than Dillon (ANOVA, F=43.6934, P<0.01) (Figure 13). Although not proven exerpimentally Dillon is a site with colder weather and that likely reflects the difference in height between trees at the two sites. DBH will be measured in summer 2020-2021.



Figure 12: Defoliation level as a function of family. Crown Damage Index method, 1-8 trees per family with two sites assessed. Linear mixed model and ANOVA analysis. Results show no difference in term of defoliation level within families or site. Nevertheless, variabilities are noticeable.



Figure 13: Height as a function of family. Crown Damage Index method, 1-8 trees per family with two sites assessed. Linear mixed model and ANOVA analysis. Results show no difference in term of defoliation level within families but the site had an effect (ANOVA, P<0.01).

CONCLUSION

Work to date has provided estimates of the resistance of individual species, families, provenances or clones to paropsine browse. We now have baseline measures of height and DBH that can be remeasured in the spring/summer of 2020 to 2021. This concurrent re-measurement of both defoliation and growth will allow us to further refine our understanding of resistance and present the first result of paropsine tolerance.

The key findings of the work by Leslie Mann in the 2019 to 2020 summer is as follows:

- Despite its own limitations the Crown Damage Index (CDI) is the best technique (tradeoff between precision and efficiency) currently available to assess defoliation of *Eucalyptus*. Tree grading is too coarse and pest counting although more quantitative is biased by the timing of sampling relative to insect development during the year. The CDI method is still time consuming and potentially subject to observer bias. As such it should be seen as a priority to develop a quantitative assessment tool and we are currently trialing image collection via a UAV for this purpose.
- CDI results show clear differences in the susceptibility of *Eucalyptus* species to defoliation at Dillon and Lissaman sites. *E. tricarpa, E. quadrangulata, E. camaldulensis,* and *E. bosistoana* were heavily defoliated, *E. macrorhyncha,* and *E. cladocalyx* had low levels of defoliation and *E. globoidea* was intermediate between these two groups.
- There was substantial range in the defoliation within a species and individual outliers had low levels of defoliation. Specific individuals may be a sampling artifact, i.e., low numbers of insects by chance, however these individuals show promise for further investigation to inform breeding decisions.
- Baseline DBH and height measurements were taken to inform future estimates of tolerance after repeated sampling. Baseline measures showed the DBH of *E. bosistoana* was smaller than *E. cladocalyx*, *E. globoidea* and *E. macrorhyncha* at the Dillon site but this was not replicated at Lissaman. The relationship between insect derived defoliation and growth will be confirmed after the 2020-2021 measurements to compare with baseline measures reported here.
- Within family variability to defoliation of *E. bosistoana* was high, in part due to the low number of replicates at the Dillon trial. The rank order of defoliation amongst families provides a guide for further investigation of optimal families for breeding decisions. Some families exhibited relatively low levels of defoliation that were comparable with that observed for *E. macrorhyncha*, and *E. cladocalyx* in the multiple species trial. These families require further assessment.
- Variability in defoliation amongst *E. bosistoana* clones was less than the intra-family variability. The rank order of defoliation amongst clones identifies those clones that are most resistant to paropsine browse. Further sampling to address tolerance is required before the best decisions can be made about which clones are likely to be least affected by paropsine browse.
- Assessment of *E. tricarpa* families showed consistently high levels of defoliation that was consistent with that observed in the multiple species trial.

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